ABSTRACT

Title of dissertation: THE DIVERSITY OF BURROWING BENTHIC INVERTEBRATES AND THEIR IMPACT ON PHOSPHORUS DYNAMICS IN AGRICULTURAL DRAINAGE DITCHES

Alan William Leslie, Doctor of Philosophy, 2014

Dissertation directed by: Professor William O. Lamp
Department of Entomology

Agriculture remains the most widespread cause of impairment of freshwater habitats, but farm lands with artificial drainage structures such as ditches have specific locations where natural physical and biogeochemical processes can be used to reduce nutrients delivered to local watersheds. Agricultural drainage ditches can also be sources of biodiversity, serving as patches of uncropped aquatic habitat that may provide a significant amount of diversity to agricultural landscapes. Macroinvertebrate communities play important roles in nutrient cycling in natural aquatic ecosystems, but to this date no information exists on the role of invertebrate communities in biogeochemical

processes occurring in ditches. The overall goal of my dissertation was to determine the structure of the aquatic macroinvertebrate community of agricultural drainage ditches, and to determine the functions these species play in nutrient cycling.

First, I performed a broad survey of aquatic macroinvertebrates in drainage ditches and related the community composition to environmental conditions. Ditches support different communities of macroinvertebrates, and community composition is correlated with physical habitat characteristics such as flow velocity (r^2=0.58) and ditch size (r^2=0.56), rather than water quality. I then measured the burrowing community of small (field) and large (collection) ditches over a year to determine how size class and seasonality affect taxonomic and functional group composition. I found small and large ditches support different taxa due to the intermittent water condition of small ditches, but both types of ditches support similar functional groups. There is limited diversity among functional feeding groups in ditches, but the majority of macroinvertebrates (101 of 140 taxa) are benthic taxa that may cause bioturbation of ditch sediments. I used microcosms to measure the effect that different burrowing species (*Ilyodrilus templetoni*, *Limnodrilus hoffmeisteri*, *Crangonyx* sp., *Chironomus decorus* S.G.) have on phosphorus dynamics between ditch sediments and water. Results show different species can increase (0.28 to 2.05 mg/L) or decrease (0.08 to 0.41 mg/L) soluble, reactive phosphorus concentrations in surface water, depending on the type of burrowing and environmental conditions. Different types of burrowers likely alter phosphorus dynamics through different mechanisms, and increasing diversity of burrowers could have non-additive effects on phosphorus uptake by ditch sediments.

THE DIVERSITY OF BURROWING BENTHIC INVERTEBRATES AND THEIR IMPACT ON PHOSPHORUS DYNAMICS IN AGRICULTURAL DRAINAGE DITCHES

By

Alan William Leslie

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2014

Advisory Committee:
Professor William O. Lamp, Chair
Professor Cerruti R. R. Hooks
Professor Bruce R. James
Professor Brian A. Needelman
Professor Margaret A. Palmer

Acknowledgements

This work would not have been possible without help throughout these years from labmates, friends, and collaborators. I would like to thank all of the landowners who let me conduct my research on their farms. Thank you to everyone who has ever been in a ditch with me catching bugs, including Sara Lombardi, Scott Berg, Libby Roberson, Sarah Au, Conor O'Leary, Brian Thompson, Melanie Stevens, Sadia Naseem, Claire Weber, and several other muddy high school interns. Thank you, Dilip Venugopal, for all the help with statistical analyses, even though I still use vegan instead of ade4. Thank you, Bob Smith, for the most comprehensive comments I have ever received on manuscript drafts. They were instrumental in getting my first paper published. Thank you to all of the collaborators on the original ditch project, Dr. David Ruppert, Kreshnik Bejleri, and Dr. Joshua McGrath. Without this initial project, I would have never formulated the dissertation objectives that I did. Finally, I would like to thank my committee members: Dr. Cerruti R. R. Hooks, Dr. Bruce R. James, Dr. Brian A. Needelman, and Dr. Margaret A. Palmer for continued feedback and ideas for improving my work, and especially my advisor, Dr. Bill Lamp for his faith and encouragement in my development academically, scientifically, and professionally.

Table of Contents

List of Tables

List of Figures

Chapter 1 - Introduction

The Clean Water Act (CWA) of 1972 was a powerful tool for protecting and restoring healthy functioning of the nation's waters, but despite this, many aquatic habitats across the nation remain degraded or are threatened by human impacts. The CWA explicitly prohibits the discharge of pollutants directly into the nation's waters, which has resulted in tight restrictions and strict regulation of point sources of pollution to waterways. Non-point sources of pollution are diffuse, and more difficult to identify and regulate, and therefore remain an important source of pollution to aquatic ecosystems. Section 303(d) of the CWA requires that every state maintain inventory of bodies of water that do not meet standards of water quality, and every state has at least some waterways listed as impaired. Of an estimated 16% of total national stream miles assessed, 44% were listed as impaired with another 3% listed as threatened with future impairment (US EPA 2009). The leading causes of impairment for the nation's waters are pathogens (25%), nutrients (18%), metals (17%), organic enrichment (16%), and sediment (16%). In the state of Maryland, nutrient enrichment is the leading cause of impairment of bodies of water, with 59% of streams and rivers impaired, and all of the remainder being threatened with future impairment (US EPA 2002b).

Agriculture and drainage ditches

Agriculture has been identified as the leading source of impairment to the nation's streams and rivers, with 38% of impaired streams having agriculture contributing to impairment (US EPA 2009). Excess nutrients carried in water draining from agricultural lands can lead to eutrophication of aquatic and coastal marine habitats. Eutrophication of aquatic habitats is the stimulation of primary productivity that results from increased

nutrient availability from human activities, and is the most widespread impairment of surface waters (Carpenter et al. 1998, Kenney et al. 2009). Increased primary productivity alters food web dynamics of aquatic habitats, resulting in drastic changes to community structure and reduced biodiversity (Withers and Jarvie 2008). This effect can be measured in streams as changes in the composition and abundance of aquatic macroinvertebrate taxa (Miltner and Rankin 1998). Non-point sources of phosphorus produce the greatest and most widespread contribution of nutrient enrichment to aquatic ecosystems, and agriculture represents the most widespread and easily recognizable source of non-point source phosphorus (U.S. Geological Survey 1999). Managing anthropogenic inputs of phosphorus to aquatic ecosystems is essential for preventing eutrophication, or for reversing its effects on aquatic communities (Conley et al. 2009).

Projections of human population growth estimate the global population to increase to over 9 billion people before levelling out within the next century (United Nations 2004). Agricultural production will have to increase in extent and intensity in order to keep pace with the nutritional requirements of this future population. Without innovative solutions for increasing the retention of nutrients on field, the expansion of agriculture is expected to increase impairment of watersheds though increased eutrophication (Tilman 1999). To meet these goals, research in sustainable agriculture will have to find solutions to protect streams while maintaining productivity.

State and federal agencies have supported the integration of sustainable practices in agriculture through different programs. The United States Department of Agriculture's Natural Resources Conservation Service (NRCS) is tasked with providing technical assistance and cost-sharing to farmers through voluntary programs that reduce impacts of

their farms to local waterways (74th United States Congress 1936). Examples of conservation practices promoted by the NRCS include planting riparian buffers along streams, managing manure stocks to prevent leaching, and taking land out of production through the Conservation Reserve Program. The NRCS often works in conjunction with state agencies to tailor their support to provide solutions that are appropriate to that region's most pressing environmental problems. For Maryland, goals for agricultural sustainability are targeted towards restoration of the Chesapeake Bay through protection of the bay's watershed. This approach to reducing effects of non-point source pollution has been effective in reducing the delivery of nutrients and sediment from fields to local watersheds (Lund 2011).

Artificial drainage structures such as subsurface tile drains and open-air ditches are specific locations where water draining from agricultural lands can be managed, and provide a unique opportunity to focus efforts to reduce delivery of agricultural pollution to local watersheds. Drainage ditches are used to control drainage of agricultural fields to prevent water stress from saturated soils and to prevent salinization of irrigated soils. These open-air drains are at the interface of agriculture and aquatic systems, and may represent the headwaters of regional watersheds. Agricultural ditches may take on different forms, from channelized, perennial streams to straight, man-made trenches that are more characteristic of linear wetlands. Estimates of the total area of farm lands within the United States with artificial drainage are as high as 44 million hectares (Pavelis 1987).

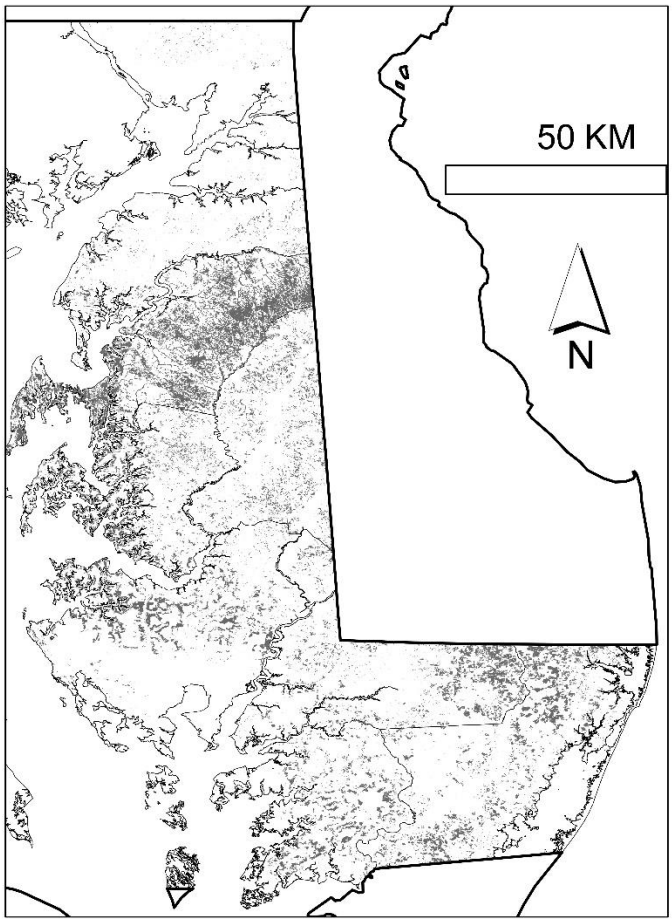
The drainage ditches studied in my dissertation were located on the Eastern Shore of Maryland, which is a portion of the peninsula east of the Chesapeake Bay. This area

falls within the coastal plain province of the state, which is characterized by flat topography and high water table with extensive surficial aquifers, creating poorly drained soils across much of the area (Denver et al. 2004). Most of the fields throughout the middle and lower regions of the Eastern Shore are only arable because of extensive networks of drainage ditches that lower the field water table below the zone of crop roots (Bell and Favero 2000). Agriculture makes up the highest percentage of land-use on the Eastern Shore, with the majority of that land being used for field crops such as corn, soybeans, and sorghum (Denver et al. 2004). Table 1-1 shows areas of land on the Eastern Shore in production of field crops that have poorly or very poorly drained soils. These values were calculated in ArcGIS as the overlapping area of soil map units with a dominant drainage class of poorly or very poorly drained using soil survey data (Soil Survey Staff, NRCS-USDA) and areas of land use that included field crops from the USDA Cropscape data (USDA National Agricultural Statistics Service Cropland Data Layer). The extent of these calculated areas (Fig. 1.1) are an underrepresentation of

Table 1-1 Total area of land with poorly or very poorly drained soils in production of field crops (corn, soy, sorghum) for Eastern Shore Counties ordered from northern to southernmost, with percent of total area of that county.

County	**field crop area (ha)**	**total area (%)**
Cecil	487	0.5
Kent	2,243	3.1
Queen Anne's	34,793	36.0
Caroline	7,420	8.8
Talbot	7,085	10.2
Dorchester	11,469	7.9
Wicomico	9,645	10.0
Worcester	13,124	10.7
Somerset	7,157	8.6

Figure 1.1 Extent of field crops grown on poorly and very poorly drained soils (represented by gray shading) on Maryland's Eastern Shore.



the total extent and application of drainage ditches on the Eastern Shore, as artificial drainage is also needed to maintain farming infrastructure, such as roads and buildings, as well as areas that may be only somewhat poorly drained.

Management practices aimed at increasing the quality of water draining from agricultural ditches manipulate conditions within the ditch to promote natural physical and biogeochemical processes (Needelman et al. 2007a). Sediment loads can be decreased by designing “two-stage” ditches that allow a natural flow course and an artificial floodplain, which reduces flow velocities and increases capacity during peak

discharge (Powell et al. 2007). Pesticide loads can be decreased by maintaining vegetation within the channel of ditches (Cooper et al. 2004). Denitrification by anaerobic bacteria can be enhanced through implementation of "biocurtains" which increase the availability of organic carbon sources to soil microbes, which increases rates of metabolism (Jaynes et al. 2008). Anaerobic conditions in ditch sediments can increase the solubility of phosphorus as surfaces of oxidized iron are reduced by anaerobic bacteria, releasing adsorbed phosphates (Venterink et al. 2002). Delivery of bioavailable forms of phosphorus can be reduced by controlling the rate of drainage from ditches, which decreases delivery of phosphorus as a function of reduced water output and allows greater residence time for phosphorus to be immobilized by adsorption or co-precipitation (Thomas et al. 1995, Moore and Kröger 2010). These conservation practices represent targeted ways of reducing export of nutrients and other pollutants from agricultural lands under artificial drainage (Strock et al. 2010).

Macroinvertebrates in drainage ditches

Another function that has been identified for drainage ditches has been their capacity to serve as sources of biodiversity for aquatic macroinvertebrates in intensely agricultural areas (Herzon and Helenius 2008). Studies of ditch macroinvertebrate communities show that some ditches are capable of supporting many of the same species as adjacent natural aquatic habitats (Verdonschot et al. 2011). In other cases, ditches may support lower diversity than natural streams and ponds, but tend to have species that are rare or absent in natural aquatic habitats (Davies et al. 2008). Drainage ditches add a new type of habitat to landscapes that can increase diversity of macroinvertebrates at the landscape scale when included with natural bodies of water (Simon and Travis 2011). Drainage ditches themselves often have high turnover of macroinvertebrate species from

one ditch to another, and therefore can support relatively high landscape-scale diversity, even if individual ditches have low diversity relative to natural streams (Williams et al. 2003).

Feeding by aquatic macroinvertebrates plays an important role in modifying rates of ecosystem functions of streams (Wallace and Webster 1996). Small, headwater streams are the site of intense biogeochemical cycling of nutrients, and processes occurring in small streams can determine the fate and delivery of nutrients to downstream waters (Alexander et al. 2000, Alexander et al. 2007, Peterson et al. 2001). Macroinvertebrates can alter nutrient flows in aquatic ecosystems by feeding on diverse food sources and exchanging nutrients between different pools either through excretion, consumption by a higher trophic level, or dispersal (Vanni 2002). Another function of streams is the transport of organic carbon from headwaters to downstream bodies of water. Headwater streams can receive large amounts of organic carbon in the form of leaf litter inputs from deciduous trees, which is then transported downstream (Cummins 1974). Macroinvertebrates can alter rates of decomposition and transport of leaf litter detritus by feeding on leaves and their associated microbes, which fragments large leaves into fine, particulate, organic matter (Wallace et al. 1982). Filter-feeding macroinvertebrates can further alter the transport of organic matter by trapping small suspended particles and depositing them as larger feces within the sediment (Malmqvist et al. 2002). Whether or not aquatic macroinvertebrates fulfill these ecosystem functions in drainage ditches is currently unknown.

Bioturbation and phosphorus dynamics

Bioturbation, or the physical disturbance of sediment, is another way that macroinvertebrates can alter rates of ecosystem functions in streams (Covich et al. 1999). Burrowing invertebrates can alter exchanges of materials between sediment and surface water, with consequences for many different functions of streams. Burrowing detritivores can alter burial rates of organic matter, making it more or less available as an energy and carbon source for sediment microbes (Creed et al. 2010, Wagner 1991). Suspension of sediment material can increase turbidity, which can increase delivery of sediments and sediment-bound materials downstream (Usio and Townsend 2004). Disturbance of sediment can also uncover dormant stages of algae and animals, which can affect rates of recruitment to the aquatic community (Yamamoto 2010).

Although some drainage ditches originated as natural stream channels that have been modified to increase water conveyance, the ecosystem functions occurring within ditches are very different from natural streams. Most ditches have no existing riparian habitat, and therefore do not receive seasonal leaf litter inputs, but rather crop residues and organic matter from mowing of ditch banks. As a result, the communities of aquatic macroinvertebrates in drainage ditches are often very different from what would be expected in natural streams in the same area. The altered community structure indicates that ditches are impaired waterways and have reduced functioning (Stephens et al. 2008, Stone et al. 2005). One of the functions that still exists in drainage ditches, and is an area of active research for ditch managements is the biogeochemical cycling of phosphorus. The functional role that macroinvertebrates play in the rates of phosphorus dynamics of drainage ditches is the main focus of my dissertation.

Ditches that are seasonally flooded can be dynamic in their tendency to sequester and release phosphorus as a function of annual fluctuations in oxidation-reduction (redox) potential. As seasonally flooded soils become saturated, redox potential decreases as microbial respiration depletes oxygen in pore spaces faster than it can be replenished by diffusion from the atmosphere. These soils can span a range in redox potential of 1000 mV over the course of a year (Vepraskas and Faulkner 2001). Intermittent flooding results in the annual reduction and translocation of iron oxides by anaerobic bacteria, and the precipitation of predominantly amorphous iron oxyhydroxides such as ferrihydrite as the soil returns to an aerobic status. Reduction of ferrihydrite in flooded soils can occur quickly, over time scales that are short enough to allow for the application of ferrihydrite as an indicator of reducing conditions within hydric soils (Rabenhorst 2006). High amounts of paracrystalline ferrihydrite can result in higher phosphate adsorption within seasonally flooded soils (Axt and Walbridge 1999). Once redox potential drops to the point where iron oxides are reduced by anaerobic bacteria, ferrihydrite is reduced to soluble Fe(II), releasing adsorbed orthophosphate into the soil solution (Young and Ross 2001). Flooded soils can act as a source of biologically available phosphorus when orthophosphate is released by reductive desorption from iron oxides (Venterink et al. 2002). The tendency for soils to release soluble reactive phosphorus during periods of flooding is problematic for utilizing ditches as temporary wetlands to mitigate nutrient pollution. Results of monitoring of different wetland soils for the removal of phosphorus have been mixed. Studies have reported seasonally flooded soils acting as net sinks (Kröger et al. 2008), sources (Venterink et al. 2002), or neither (Kovacic et al. 2000).

Seasonally flooded ditches may also vacillate between acting as a source and a sink for phosphorus during different times of the year (Nguyen and Sukias 2002).

Adsorption of orthophosphate to iron oxide coatings is not the only process that affects transformations of phosphorus in seasonally flooded soils. Variability in rates of phosphorus uptake by different ditches is likely due to differential rates of other mechanisms driving the immobilization and release of dissolved phosphorus. Orthophosphate released by the reduction of ferrihydrite may react with dissolved Fe (II) to precipitate ferric iron phosphates ($Fe_3(PO_4)_2$) (Chacon et al. 2005). Dissolved phosphorus may also continue to form complexes with species that are not redox sensitive, such as aluminum oxides, calcium, and organic matter (Richardson 1985, Bruland and Richardson 2006, Chacon et al. 2005, Sharpley et al. 2007). Mineralogy and particle size distribution of the soil can affect rates of phosphorus immobilization, as soils with higher clay contents have more surface area available to complex orthophosphate (Sharpley et al. 2007). Periodic dredging of ditches to maintain hydraulic conveyance increases phosphorus release to overlying water, and decreases phosphorus adsorption (Smith and Pappas 2007). Phosphorus may exist in solution as organic forms, including cellular constituents, such as DNA and RNA, phospholipids from cellular membranes, and ATP, or inositol hexaphosphate (phytic acid), which is a phosphorus-storing molecule produced by plants (Lott et al. 2000, Molles 2012). Anaerobic reduction of iron oxide coatings on mineral particles can also increase the activity of phosphatase enzymes in soil solution, and could result in an increase in labile phosphorus being released from insoluble organic pools (Chacon et al. 2005).

Bioturbation has been proposed as a way that macroscopic organisms can affect rates of release of phosphorus contained within sediments, and may be important for determining phosphorus dynamics in drainage ditches (Krantzberg 1985). The physical disturbance of soils by infaunal organisms (also known as pedoturbation) has also been proposed as a way that macroscopic organisms may affect pedogenic processes that determine rates of phosphorus transformation in ditch soils (Needelman et al. 2007b, Vaughan et al. 2008). The physical result of bioturbation of ditch sediments and soils is the mixing of pore water and overlying water, alteration of the physical structure of ditch substrate, and the translocation of labile organic matter (Mermillod-Blondin and Rosenberg 2006). These physical effects of bioturbation in turn impact biotic and abiotic processes in ditch soils and sediments that determine rates of sequestration and release of phosphorus.

Bioturbation physically mixes soil pore water with overlying water, moving dissolved phosphorus faster than by diffusion alone (Mermillod-Blondin and Rosenberg 2006). The invasive red swamp crayfish *Procambarius clarkii* can increase rates of flux of dissolved phosphorus from soil and sediment pore spaces to overlying water by physically mixing soil materials, releasing pore water high in dissolved phosphorus to the overlying water (Angeler et al. 2001). Studies of aquatic oligochaete worms suggest that bioirrigation, or the movement of water through animal burrows also increases the movement of nutrients from sediments to the overlying water (Mermillod-Blondin et al. 2008). Bioirrigation by different insect larvae moves dissolved oxygen deeper within the sediment profile, and can alter redox chemistry associated with nutrient transformations (De Haas et al. 2005, Gallon et al. 2008).

Changes to the physical structure of ditch sediments can alter the flow of water through sediment layers. Burrows created by aquatic oligochaetes increase the hydraulic conductivity of sediments (Nogaro et al. 2006). Large burrows, or biopores, can drastically alter the flow path of water infiltrating agricultural fields, bypassing any interaction between the water and soil particles (Guzman et al. 2009). Limiting the interaction of subsurface water with soil particles may decrease the ability of ditch sediments to immobilize dissolved phosphorus by adsorption to surfaces.

Increased micro-scale habitat heterogeneity is another mechanism proposed for the increased flux of phosphorus, as infaunal organisms create localized deposits of labile organic matter available to microbes driving many of the biochemical transformations of phosphorus within the soil (Stockdale et al. 2009). Availability of labile carbon promotes bacterial growth and assimilation of available phosphorus that becomes immobilized in bacterial biomass.

To understand the impact of bioturbation on phosphorus dynamics in drainage ditches, the benthic invertebrate community disturbing ditch substrate must be identified. Ditches on the Eastern Shore of Maryland have uniform substrate, with no hard surfaces and little heterogeneity in channel morphology. This limits the habits of aquatic invertebrates to crawling along the surface, swimming within the water column, or burrowing within the soil or sediment. Benthic macroinvertebrate communities of agricultural drainage ditches tend to be dominated by burrowing taxa such as oligochaete worms and chironomid larvae (Feldman et al. 2010, Werner et al. 2010). Some ditches are dominated by aquatic isopods *Caecidotea spp*., which may produce burrows only as ditches begin to dry during warmer months (Smith 2001). Physical differences in the

types of burrows created are taxon-specific, and could result in different effects on phosphorus cycling (Charbonneau and Hare 1998, Nogaro et al. 2006). Since some ditches tend to be dry during the summer months, community composition as well as effects of bioturbation likely change as a function of seasonal fluctuations in water table and temperature.

Dissertation overview

The goal of my dissertation research was to characterize the community of aquatic macroinvertebrate organisms inhabiting agricultural drainage ditches, and to investigate the effects of their burrowing activity on the fate of phosphorus within drainage ditches. My research determined the composition of the macroinvertebrate community inhabiting agricultural drainage ditches and identified the species responsible for bioturbation. In addition, my research determined the effect that bioturbation has on the availability of dissolved orthophosphates, and investigated mechanisms involved in producing that effect. The results of this work will help to provide a better understanding of the dynamics of phosphorus within drainage ditches, and has implications for designing drainage ditches that function better to immobilize phosphorus and to reduce inputs to downstream waters.

My dissertation is arranged as the following four chapters. Chapter two is entitled, "Environmental factors structuring benthic macroinvertebrate communities of agricultural ditches in Maryland." In this chapter, I show how communities of benthic macroinvertebrates inhabiting drainage ditches vary in abundance and composition in response to environmental variables. Chapter three is entitled, "Patterns of burrowing invertebrate communities in small and large agricultural drainage ditches." In this

chapter, I describe the taxa, density, and functional group composition of macroinvertebrates on and in ditch sediments, and how composition and abundance varies between small (field) and large (collection) ditches, and across seasons. My third chapter is entitled, "Burrowing macroinvertebrates alter phosphorus dynamics in drainage ditch sediments." In this chapter, I show how the activity of burrowing benthic invertebrates affects the movement of phosphorus from ditch sediments to overlying waters. My fourth and final chapter is entitled "Conclusions: The structure and function of ditch benthic invertebrate communities." In this chapter I pull together findings from all preceding chapters to formulate overall conclusions on the role of aquatic macroinvertebrates on ecosystem processes in drainage ditches.

Chapter 2 - Environmental factors structuring benthic macroinvertebrate communities of agricultural ditches in Maryland[1]

ABSTRACT Agricultural drainage ditches are man-made structures used to optimize soil hydrology for crop production and secondarily have been co-opted as a tool to manage the quality of water draining from agricultural lands. We investigated the relationship between the aquatic macroinvertebrate community and environmental variables associated with physical and biogeochemical processes that affect water quality. Aquatic macroinvertebrates were sampled along with physical and chemical measures of the soil and water from 29 agricultural drainage ditches on the Eastern Shore of Maryland. Cluster analysis and multivariate ordination showed that ditches that had higher flow velocities supported communities of lotic invertebrates (ie. *Stenelmis*, *Prosimulium*) versus those that had properties of linear wetlands, which supported communities of lentic invertebrates (ie. Oligochaeta, *Caecidotea*). Taxon richness varied from 4 to 31 taxa per ditch, and was higher within ditches that had higher flow velocities. Small ditches had low diversity, but may have provided refugia from fish predators. Macroinvertebrate communities did not show a significant linear relationship with water quality or with nutrient concentrations within the soil or water. The addition of flow-control structures designed to improve the quality of water draining from agricultural lands may decrease the quality of ditches as habitat for certain aquatic macroinvertebrates. Management decisions for drainage ditches may consider tradeoffs

[1] This chapter has been published as: Leslie, A. W., R. F. Smith, D. E. Ruppert, K. Bejleri, J. M. Mcgrath, B. A. Needelman, and W. O. Lamp. 2012. Environmental factors structuring benthic macroinvertebrate communities of agricultural ditches in Maryland. Environmental Entomology 41:802–812.

between the benefits of ditches as a source of biodiversity and as a tool for improving water quality.

Introduction

Drainage is essential for maintaining productive agricultural areas around the globe. The International Commission on Irrigation and Drainage estimates that globally, 190 million hectares of agricultural lands are drained artificially, with most of that land in the Americas (65 Mha), Asia (58 Mha), and Europe (47 Mha) (ICID 2010). In the United States, agricultural drainage networks often comprise subsurface drains that empty into small in-field ditches, which feed into larger collection ditches running between individual properties (Pavelis 1987). Drainage ditches are at the interface between agriculture and aquatic ecosystems, and replace the natural headwaters of regional watersheds.

These engineered waterways provide habitat for species of plants, fish and macroinvertebrates and contribute to landscape scale biodiversity (Armitage et al. 2003, Davies et al. 2008, Herzon and Helenius 2008). Ditches may have lower diversity than other types of aquatic habitats, but they can provide habitat for species not found in larger, perennial bodies of water (Williams et al. 2003). Drainage ditches may increase taxonomic richness of invertebrates in stream networks by increasing habitat heterogeneity relative to natural stream networks without ditches (Simon and Travis 2011). Studies of invertebrate communities in drainage ditches in fens of England have discovered invertebrate species of conservation value (Painter 1999). Native fish species use intermittently flowing drainage ditches as a refuge during seasonally stressful times and also as sheltered breeding grounds (Colvin et al. 2009).

Modern surface ditches are designed to minimize transport of sediments and chemicals from agricultural land into surface waters. Agriculture represents a widespread non-point source of pollution to aquatic ecosystems, but drainage ditches represent a specific location where loads of sediment, nutrients, and pesticides can be targeted for mitigation (Cooper 1993, Skaggs et al. 1994). Biogeochemical and physical processes occurring within drainage ditches are being used as a tool for improving the quality of water leaving agricultural fields and entering local watersheds (Needelman et al. 2007b). Managing the flow regimes of drainage ditches can promote physical processes that prevent erosion of sediments and support oxidation/reduction (redox) reactions that may decrease nutrients draining from agricultural ditches (Needelman et al. 2007a, Strock et al. 2007). Installation of structures, such as weirs or flashboard risers, that control discharge from ditches limits the export of nutrients in the form of nitrogen and phosphorus to downstream habitats (Skaggs et al. 1994, Thomas et al. 1995). These flow-control structures also increase residence time of nutrient-rich waters within ditches, which promotes increased rates of denitrification (Penn et al. 2010). The United States Department of Agriculture - Natural Resources Conservation Service (USDA-NRCS), in conjunction with state agencies, provides funding for ditch management aimed at reducing the load of nutrients exported from agricultural fields to streams and rivers (http://www.nrcs.usda.gov/programs/, http://www.mda.state.md.us/pdf/pda.pdf). This has led to widespread installation of flow-control structures through much of the Ohio and Mississippi River valleys and the Chesapeake Bay watershed that reduce nutrient export from agricultural fields, while at the same time increasing yields for farmers (Fouss and Sullivan 2009, Penn et al. 2010).

Management of drainage ditches to increase the quality of water draining from agricultural lands involves manipulation of ditch habitat characteristics that could increase or decrease quality of ditches as habitat for aquatic organisms. Riparian habitat (Moore and Palmer 2005) and benthic habitat (including hydrology) (Verdonschot and Higler 1989, Painter 1999, Davis et al. 2003, Stone et al. 2005, Stephens et al. 2008) are the factors most closely associated with patterns in aquatic invertebrate community composition of agriculturally impacted streams and ditches. Increased sedimentation behind flow-control structures could change the physical structure of substrate encountered by benthic macroinvertebrates. Changing vegetation structure by mowing or dredging within ditches alters substrates and food resources used by macroinvertebrates. Aquatic macroinvertebrate species richness is positively related to dissolved oxygen concentration, and species composition is related to pH in ditches (Verdonschot and Higler 1989, Werner et al. 2010). Controlling drainage to promote microbially mediated redox transformations of nutrients will decrease flow velocities and promote development of anaerobic sediments in ditch habitats.

In the present study, we sought to determine what communities of invertebrates are present in drainage ditches, and investigated patterns between aquatic macroinvertebrate communities and the physical and chemical parameters that are managed to promote biogeochemical processing of nutrients in agricultural drainage ditches. Our hypothesis is that physical, benthic habitat characteristics and water chemistry parameters determine the composition of aquatic macroinvertebrate communities of drainage ditches. Physical alterations to ditches related to management for drainage and nutrient mitigation will affect the suitability of ditches as habitat for

different invertebrate communities. The results will help to determine what invertebrate taxa colonize ditches, and whether management for nutrient removal might conflict with management for a diverse aquatic community within ditches.

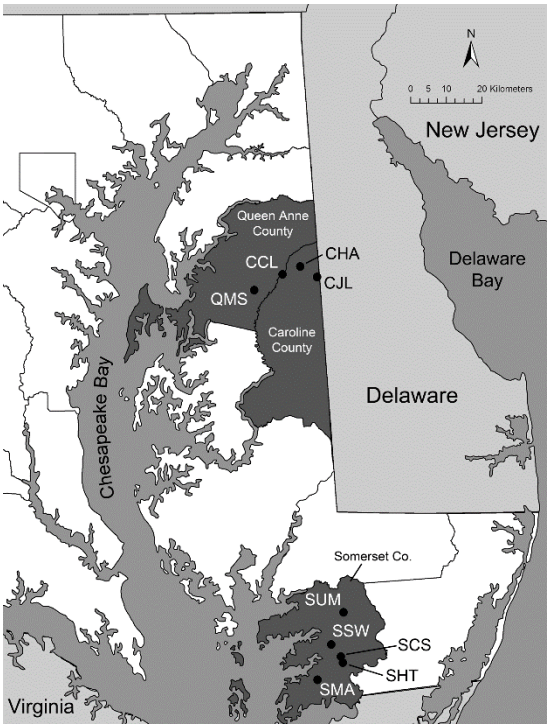
Materials and Methods

Site description

The study was conducted on drainage ditches located on the Eastern Shore of Maryland, which is the portion of Maryland east of the Chesapeake Bay (Fig. 2.1). Agriculture makes up the highest percentage of land-use of the area (48%), with the majority of that land being used for crops such as corn and soybeans (Denver et al. 2004). The middle and lower regions of the Eastern Shore are within the Coastal Plain physiographic province, and are characterized by flat topography and poorly drained soils. Most fields throughout the middle and lower regions are only arable because of drainage structures that lower the field water table below the zone of crop roots (Bell and Favero 2000, Denver et al. 2004). Most of the farms in this area lie within the watershed of the Chesapeake Bay, which suffers from negative impacts of eutrophication caused in part by nutrient losses from agriculture (Phillips 2007). For this reason, some drainage ditches on the Eastern Shore of Maryland are designed for mitigation of nutrient inputs to receiving waters.

A total of 29 ditches were sampled between 23 Feb and 15 Mar 2008. Selected farms were located in three counties, representing the middle (Queen Anne and Caroline Counties) and lower (Somerset County) Eastern Shore. Ditches were chosen to represent a wide range of environmental conditions, including ditch size, flow velocity, substrate type, and presence of flow-control structures. Twenty-three ditches were located within actively farmed fields, 18 ditches were within fields that were either planted with cover

Figure 2.1 Map of the Eastern Shore of Maryland showing Maryland counties and bordering states. The dark shaded areas indicate the three counties that were sampled. Individual farms are indicated by black circles and are labeled with respective site codes.



crops (winter wheat, *Triticum aestivum* L.) or retained crop residues (corn, *Zea mays* L.), while five were adjacent to tilled fields. Of the ditches not within actively farmed fields, three ditches were located within fallow fields (CCL-1, 5, 6), and three ditches were adjacent to a site recently converted from agricultural lands to wetlands (CJL-1, 2, 3).

We collected invertebrate and environmental samples concurrently to reflect each ditch as a single sample unit in our analysis. At each ditch, a 50 m reach was selected just upstream (~10 m) of either the confluence with its receiving body, or the flow-control structure if present. Invertebrate collection, soil and water sampling, and ditch physical characteristics were all done within this reach.

Environmental measurements

Water chemistry and flow were measured at a single point at the downstream end of the sampling reach. A single 500 mL water sample was taken from each ditch before water was disturbed with other sampling, transported on ice, and then frozen until analyzed for total nitrogen, total phosphorus, and total solids (QuikChem 8500, Lachat instruments, Colorado, USA). Dissolved oxygen, pH, and specific conductivity were measured on site using handheld meters (YSI 55/63, YSI inc., Ohio, USA). Flow velocity was measured at the center of the downstream end of the reach using a Flow Mate Model 200 portable flowmeter (Marsh McBirney inc., Colorado, USA).

Ditch physical structure and soil chemistry were measured at three points along the reach by dividing the reach into three equal strata, and choosing a point among 1 m increments within each stratum using a random numbers table. Ditch geometry was assessed by measuring ditch depth from bankfull height to ditch bottom along transects across the three sampling points. Cross sectional area and maximum depth, as well as measures of the average wetted width and maximum water depth within the ditch were calculated from these measurements. Percentages of vegetative and detrital cover were estimated visually across the wetted width of the ditch, along 1 m length of the ditch. Soil cores were extracted using a metal ring 15.2 cm in diameter and 5 cm in height pushed into the soil. Samples were separated by horizon in the field, transported on ice to the laboratory where they were stored at 4 ºC until analyzed for bulk density as well as total carbon and nitrogen (Tru Spec, Leco, Michigan, USA). Measurements of soil pH and redox potential were made at the soil surface and 2.5 cm below the surface using six platinum-tipped electrodes and a Calomel (Hg/HgCl) reference electrode connected to a handheld voltmeter modified according to Rabenhorst (2009). A correction factor of 251

mV was added to the measured voltages so that reported redox potentials are relative to a standard hydrogen electrode (Vepraskas and Faulkner 2001). We plotted mean redox potentials against soil pH from each ditch along with a technical standard for soil redox potential, which is an empirically determined line that divides conditions where redox reactions are predominantly oxidizing from conditions where redox reactions would be predominantly reducing, adjusted for pH (Vasilas et al. 2010).

Macroinvertebrate collection

Invertebrates were collected from ten points selected by dividing the sampling reach into 1 m increments and choosing points from a table of random integers 0 – 50. At each point, two successive 1 m long sweeps were made with a D-frame net (0.05 m^2 opening, 500 μm mesh) to collect invertebrates within the substrate and water column. The D-frame net was chosen for its ability to capture a diverse assemblage of aquatic invertebrate species (Turner and Trexler 1997). Individual sweeps were combined into a composite sample for the entire ditch, and preserved using 80% ethyl alcohol. In the laboratory, samples were rinsed in stacked 4 mm and 500 μm sieves to remove coarse plant material while retaining macroinvertebrates and fine debris. The remaining sample material retained by the 500 μm sieve was sub-sampled by spreading the material onto a numbered 7×7 mesh grid of 4×4 cm squares and randomly selecting squares for sorting. Macroinvertebrates were removed from sample material under a dissecting microscope. Subsequent sub-samples were taken until a minimum of 300 macroinvertebrates were recovered, or until the entire sample had been sorted. Total abundance of macroinvertebrates within samples was extrapolated based on the amount of sample material sorted.

All insects and crustaceans were identified to genus, except for some Diptera larvae of the suborder Brachycera, and early instar insect larvae, which were identified to family. Samples containing large numbers of larvae in the family Chironomidae (order Diptera) were further sub-sampled so that 20% of each morphotype were slide-mounted and identified to genus. Mollusks were identified to the family level, and aquatic Oligochaeta were not identified beyond the subclass level. Merritt et al. (2008) and Covich and Thorp (2001) were used to assign taxa to functional feeding groups (FFG) and groups based on habits of that taxon (e.g. swimming versus burrowing) to determine functional roles of invertebrates within ditch habitats.

Data analyses

Hierarchical cluster analysis was used to determine whether ditch invertebrate communities could be divided into distinct groups based upon differences in invertebrate community composition. A Bray-Curtis distance matrix was calculated from log(x+1) taxon counts, and clusters were formed using Ward's agglomerative method. Taxa associated with groups formed by cluster analysis were determined using IndVal scores, which are calculated based on fidelity and relative abundance of taxa within groups created by cluster analysis. The number of groups that resulted in the maximum sum of significant IndVal scores was used as the stopping point for forming groups of sites from cluster analysis (Dufrene and Legendre 1997). Taxon richness, Shannon diversity, Simpson's dominance, total abundance of organisms per sample, and Shannon diversity of FFG were calculated for groups formed by cluster analysis to further describe differences between groups.

Site groupings based on cluster analysis were compared to a plot of sites in multivariate space. Non-metric multidimensional scaling (NMDS) was used to create a multivariate ordination using the same distance matrix calculated for cluster analysis. Ordinations were constructed using multiple runs with random starting configurations. All benthic habitat and water quality variables (Table 2-1) were fitted to the final NMDS ordination to determine patterns between clusters of ditch macroinvertebrate communities and environmental parameters. Environmental variables were checked for colinearity using linear regressions, and when explanatory variables were found to be collinear, only the variable with the higher r^2 value was retained. Significance of the relationship between environmental vectors and the ordination of sites was determined by Monte Carlo permutation ($\alpha = 0.05$). Significant environmental variables were then used in linear regressions with FFG diversity to determine changes in the diversity of feeding habits along environmental gradients.

Statistical analyses were all performed using R v. 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria). Cluster analysis and NMDS were performed using the package vegan v. 1.17-2, and IndVal scores were calculated using the package labdsv v. 1.4-1.

Results

Environmental measurements

The physical and chemical attributes of the ditches are summarized in Table 2-1. Ditch size varied from small field drains with a ditch depth from bankfull height of 0.38 m and a maximum cross-sectional area of 0.53 m^2 to larger collection ditches with a depth of 1.64 m and maximum cross-sectional area of 6.71 m^2. Most ditches contained an abundance of detritus in the form of coarse particulate organic matter within the channel,

with greater than half of sites having coverage >90%. Cover by rooted vegetation showed greater variation and ranged between 0 – 100% between ditches. All ditches were periodically dry during the previous summer, but contained water at the time of sampling, with a maximum depth ranging from 0.03 to 0.37 m. Flow velocity ranged from undetectable in stagnant ditches to 0.20 m s^{-1} in ditches more typical of channelized streams. All ditch water was acidic, with a mean pH of 5.4 (range 4.6 – 6.4). Mean specific conductivity was 217.4 µS cm^{-1} (range 34.5 – 448.1 µS cm^{-1}) and total solids had a mean of 2.5 g L^{-1} (range 0.03 – 11.6 g L^{-1}). Dissolved oxygen also varied between ditches, but most ditches had relatively high DO, with 12 of 29 ditches being near or even beyond saturation.

Six ditches had redox states below the technical standard (anaerobic) for both depths, three ditches were below the technical standard at a single depth, and 20 were above (aerobic) at the time of measurement (Fig. 2.2). Ditch soils often exhibit aerobic conditions at the soil-water interface and anaerobic conditions at depth (Needelman et al. 2007a). The predominance of aerobic conditions at the 0 and 2.5 cm depths at these sites indicate that we likely took our measurements within the soil zone influenced by the soil-water interface.

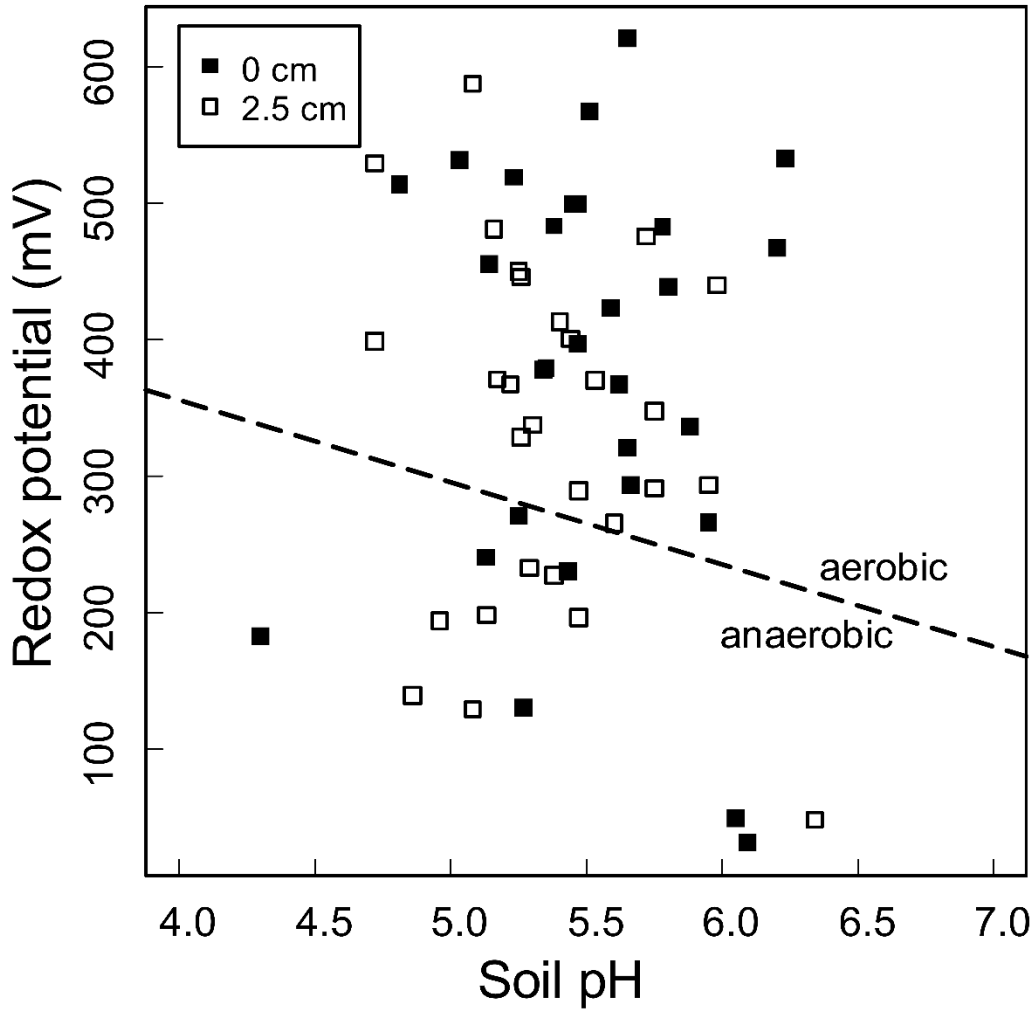
Table 2-1 Benthic habitat and chemical measurements of 29 sampled ditches.

	D.O.	Sp. Cond.	Water pH	Total solids	Water tot. N	Water tot. P	Redox 0cm	Soil pH 0cm	Redox 2.5cm	Soil pH 2.5 cm
Ditch	% sat.	µS/cm	-	g/L	mg/L	mg/L	mV	-	mV	-
CCL-1	87	165	5.7	0.26	10.4	0.26	467	6.2	370	5.5
CCL-2	142	266	6.2	0.05	13.4	0.47	499	5.5	439	6
CCL-3	34	213	5.5	0.1	1.73	0.26	499	5.5	529	4.7
CCL-4	8	249	5.9	0.12	10.3	0.08	230	5.4	232	5.3
CCL-5	88	234	4.8	0.12	10.3	0.1	483	5.4	475	5.7
CCL-6	74	136	5.5	0.06	1.12	0.18	31	6.1	48	6.3
CHA-1	57	115	4.6	0.07	5.62	0.16	378	5.4	400	5.4
CHA-2	48	129	5.3	0.06	5.43	0.48	378	5.3	265	5.6
CJL-1	44	49	4.7	0.11	7.62	0.75	455	5.1	293	5.9
CJL-2	101	170	5.6	0.18	9.37	1.61	336	5.9	194	5
CJL-3	58	75	5.3	0.03	0.81	0.08	271	5.3	129	5.1
QMS-1	148	163	5.5	0.08	9.05	0.13	533	6.2	398	4.7
QMS-2	108	207	6.4	0.1	3.52	0.1	620	5.7	652	5.6
QMS-3	121	234	5.6	0.31	6.9	0.1	567	5.5	481	5.2
SCS-1	110	254	5.3	0.16	1.75	0.26	130	5.3	139	4.9
SCS-2	29	243	4.9	0.16	0.97	0.12	49	6	198	5.1
SCS-3	50	35	5.4	0.2	10.3	0.76	367	5.6	328	5.3
SHT-1	44	229	5.5	0.15	5.55	0.08	293	5.7	227	5.4
SHT-2	106	309	4.7	1.92	8.34	3.09	531	5	445	5.3
SHT-3	114	342	5.8	0.21	4.94	0.4	266	5.9	291	5.7
SMA-1	94	304	5.5	11.36	2.66	0.69	321	5.6	337	5.3
SMA-2	34	208	5.2	11.6	1.5	0.23	397	5.5	367	5.2
SMA-3	71	268	5.7	10.67	1.79	0.23	439	5.8	413	5.4
SSW-1	16	156	5.4	0.53	4.68	0.84	519	5.2	450	5.2
SSW-2	60	143	5.7	0.27	2.75	0.77	240	5.1	289	5.5
SSW-3	100	167	5.6	0.1	1.08	0.19	182	4.3	196	5.5
SUM-1	138	369	5.5	11.46	4.66	0.09	514	4.8	587	5.1
SUM-2	70	448	5.1	10.85	3.91	0.79	423	5.6	348	5.8
SUM-3	99	424	5.6	11.22	27.7	0.87	483	5.8	371	5.2
Mean	78	217	5.4	2.5	6.1	0.48	376	5.5	341	5.4
(S.D.)	(38)	(101)	(0.4)	(4.5)	(5.5)	(0.62)	(153)	(0.4)	(140)	(0.4)

Table 2-1 continued

	Soil Tot. C	Soil tot. N	Bulk density	Plant cover	Detrit cover	Flow veloc.	Cross section	Ditch depth	Water depth	Flow control
Ditch	ppt	ppt	g/cm^3	%	%	m/s	m^2	m	m	Y/N
CCL-1	206	13.8	0.17	17	100	0	3.1	1	0.08	Y
CCL-2	77	4.9	0.45	60	100	0.02	4.7	0.9	0.17	N
CCL-3	74	5.2	0.16	23	100	0.02	4.9	1.6	0.28	Y
CCL-4	71	4.7	0.2	2	67	0	3.2	1.3	0.37	Y
CCL-5	14	1	0.48	0	100	0.02	4.6	1.3	0.09	Y
CCL-6	4	0.3	1.12	1	100	0	5.2	1.6	0.11	Y
CHA-1	49	3.4	0.52	40	100	0.03	1.8	0.7	0.13	N
CHA-2	162	11.9	0.17	17	100	0.02	3.7	1	0.28	N
CJL-1	185	11.1	0.16	15	100	0	3.3	0.9	0.18	Y
CJL-2	198	11.4	0.28	100	63	0.03	6.7	1.6	0.29	N
CJL-3	164	9.1	0.34	5	80	0	2.2	0.8	0.17	N
QMS-1	51	3.9	0.43	59	100	0.05	4.8	1.6	0.11	Y
QMS-2	4	0.4	1.62	2	38	0.2	3.4	1.1	0.21	N
QMS-3	3	0.3	1.9	13	35	0.06	2.5	0.9	0.09	N
SCS-1	30	2.3	0.7	33	95	0.02	0.5	0.4	0.08	Y
SCS-2	30	2.7	1.08	50	100	0.02	0.9	0.6	0.19	N
SCS-3	72	5.1	0.48	33	100	0	0.8	0.4	0.06	N
SHT-1	23	1.8	0.85	5	67	0	0.7	0.4	0.08	Y
SHT-2	29	2.2	0.46	37	97	0.03	1.6	0.8	0.06	N
SHT-3	99	7.5	0.39	27	100	0.03	1.5	0.6	0.14	N
SMA-1	42	3.4	0.56	10	93	0	1.4	0.7	0.22	Y
SMA-2	45	3.2	0.79	62	48	0	1.6	0.8	0.04	N
SMA-3	21	1.5	0.8	83	77	0	1.5	0.7	0.03	N
SSW-1	25	2.1	0.97	22	83	0	1.6	0.7	0.05	Y
SSW-2	33	2.8	0.68	0	80	0	1.2	0.6	0.11	N
SSW-3	27	2.3	0.81	5	83	0	2	0.9	0.22	N
SUM-1	28	2.1	1.36	33	40	0.02	2.3	1	0.13	Y
SUM-2	132	8.9	0.39	5	87	0.02	0.7	0.5	0.08	Y

Figure 2.2 Plot of average redox potential measures and soil pH measured at each ditch. Dark squares are measures taken at the soil surface and open squares are measurements from a depth of 2.5 cm. The line (Redox = 595 – 60 × pH) represents the division between soils where iron would be oxidized (points above the line) and reduced (points below the line) as determine by National Technical Committee for Hydric Soils.



Mean total nitrogen concentration of ditch water was 6.1 mg L^{-1} (range 0.81 - 27.7 mg L^{-1}) and average total phosphorus of ditch water was 0.48 mg L^{-1} (range 0.08 - 3.09 mg L^{-1}). In comparison, the average nutrient concentrations of surface waters of the area in early spring are 3.5 mg L^{-1} for total nitrogen and 0.1 mg L^{-1} for total phosphorus (Denver et al. 2004). The mean total carbon concentration of the upper 5 cm of soil was

67 g C kg^{-1} soil (range 2.6 - 206 g C kg^{-1} soil), while mean total soil nitrogen was 4.6 g N kg^{-1} soil (range 0.29 - 13.8 g N kg^{-1} soil).

Macroinvertebrate community

A total of 9081 individual organisms were identified from sub-samples of all benthic invertebrate samples, representing 85 invertebrate taxa (Table 2-2). Six of the seven Odonata, Ephemeroptera, Plecoptera, and Trichoptera taxa identified to family were represented by a single individual, or multiple individuals recovered from a single ditch, and therefore the use of family level identifications should not have underestimated overall taxa richness for these groups compared to those taxa identified to genus. Abundance of organisms per sample was estimated by extrapolation of the proportion that was subsampled, and varied from 58 to 10,780 individuals per sample. Taxon richness varied from 4 to 31 taxa with a mean of 13.9 taxa identified per ditch. Insects represented the majority of the invertebrate taxa, with 75 taxa from seven different orders. Diptera was the most diverse insect order, with 38 different taxa recovered from the ditches. Non-insect taxa had a greater total abundance than insects, representing three quarters of all individuals recovered. Among the non-insect taxa, isopods of the genus *Caecidotea* and aquatic oligochaete worms were the most numerous and represented the dominant taxon in 21 ditches. The majority (80.7%) of individuals collected across all ditches were detritivores (collector/gatherer feeding group). Other feeding groups represented were predators (7.6%), filter feeders (5.9%), scrapers (3.7%), shredders (2.1%), and herbivores (0.1%). The dominant habits of ditch invertebrates were sprawling on the sediment surface (54.0%) and burrowing within sediments (38.5%). Other habits represented included swimming within the water column (5.2%), clinging to hard surfaces (2.2%), and climbing emergent vegetation (0.2%).

Table 2-2 Macroinvertebrate taxa sampled from ditches.

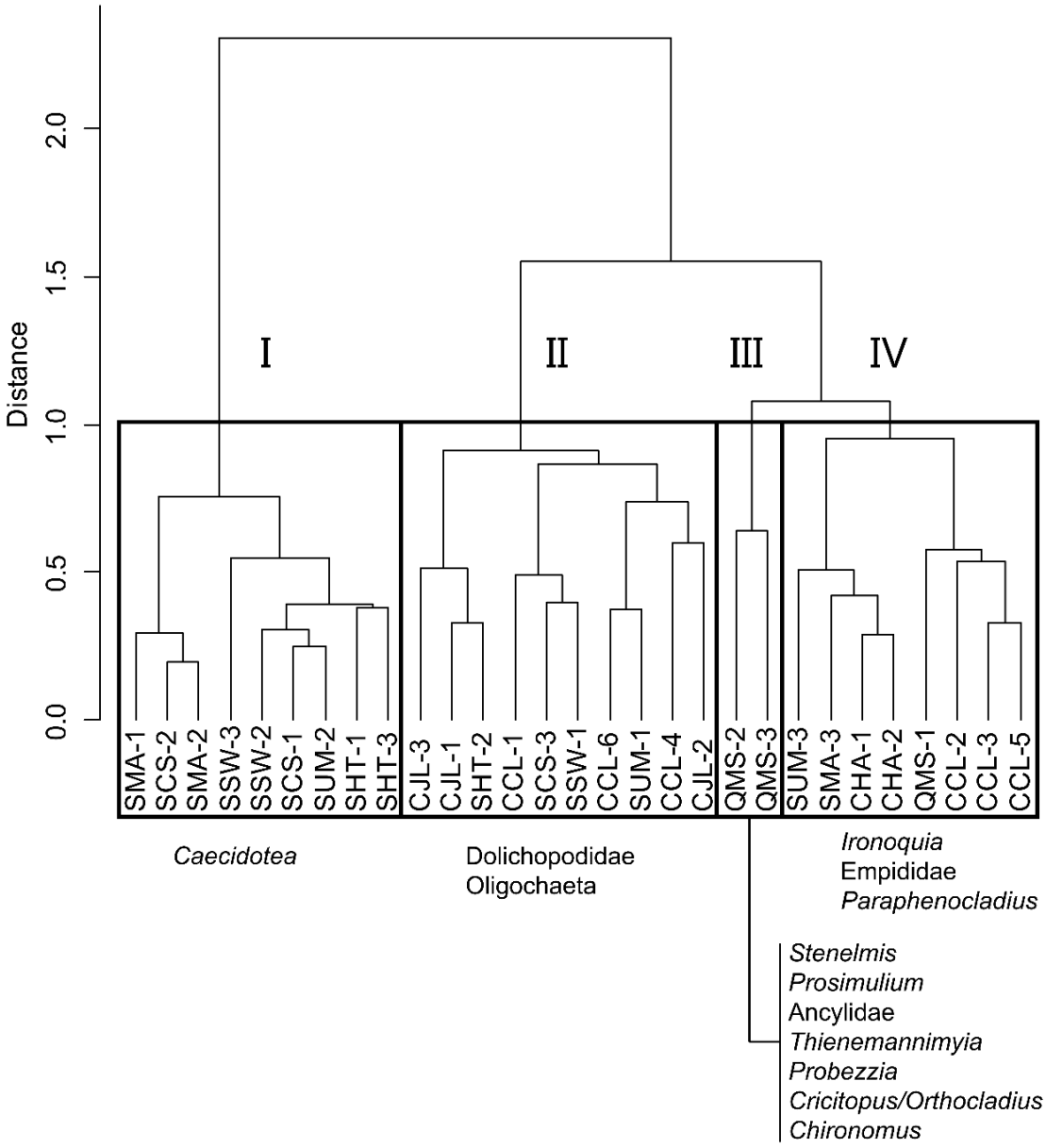
Taxon					Frequency	Total
Phylum	**Class**	**Order**	**Family**	**Genus**	**(# ditches)**	
Arthropoda	Hexapoda	Odonata	Gomphidae	*Dromogomphus*	1	2
			Libellulidae	*Libellula*	1	1
			Coenagrionidae	-	2	4
				Amphiagrion	1	1
				Argia	2	2
		Ephemeroptera	Baetidae	-	1	3
			Ephemerellidae	-	1	14
			Caenidae	*Caenis*	1	3
			Heptageniidae	-	1	1
		Plecoptera	Leuctridae	*Leuctra*	1	1
			Taeniopterigidae			
				Taeniopteryx	1	4
			Chloroperlidae	-	1	3
		Trichoptera	Limnephilidae	*Ironoquia*	11	110
				Limnephilus	5	7
			Polycentropodidae			
				-	1	1
			Hydropsychidae			
				Hydropsyche	1	2
				Cheumatopsyche	1	2
		Megaloptera	Corydalidae	*Chauliodes*	1	1
		Coleoptera	Haliplidae	*Peltodytes*	2	2
			Elmidae	*Dubiraphia*	1	7
				Microcylloepus	1	1
				Stenelmis	2	19
			Scirtidae	*Cyphon*	1	1
			Hydrophilidae	*Hydrochus*	2	3
				Tropisternus	2	2
				Berosus	2	2
				Paracymus	3	4
				Enochrus	1	2
			Dytiscidae	*Copelatus*	3	6
				Hydroporus	3	3
				Neoporus	10	43
				Uvarus	1	1
				Hydrovatus	1	1
				Agabus	15	61
			Noteridae	*Hydrocanthus*	1	4
				Suphisellus	1	1
		Diptera	Chironomidae	*Thienemannimyia*	3	82
				Larsia	3	15
				Zavrelimyia	2	15
				Limnophyes	14	281
				Tvetenia	11	237

Table 2-2 continued

Taxon					Frequency	Total
Phylum	**Class**	**Order**	**Family**	**Genus**	**(# ditches)**	
				Smittia	10	159
				Paraphenocladius	5	49
				Psilometriocnemus	1	54
				Cricotopus/Orthocladius	13	272
				Diplocladius	4	31
				Eukiferellia	2	11
				Zalutschia	1	5
				Tanytarsus	2	10
				Rheotanytarsus	1	5
				Polypedilum	11	299
				Tribelos	1	10
				Endotribelos	1	5
				Dicrotendipes	1	5
				Chironomus	1	10
			Culicidae	*Aedes*	1	10
			Chaoboridae	*Mochlonyx*	2	6
			Tipulidae	*Tipula*	16	65
				Ormosia	2	5
			Ceratopogonidae			
				Forcipomyia	1	1
				Probezzia	4	21
				Bezzia/Palpomyia	5	14
				Culicoides	4	38
				Ceratopogon	3	8
				Alluaudomyia	2	4
				Dasyhelea	1	1
			Psychodidae	*Pericoma*	3	6
				Psychoda	1	1
			Simuliidae	*Prosimulium*	2	148
			Stratiomyidae	*Odontomyia*	1	2
			Tabanidae	*Tabanus*	4	10
			Dolichopodidae	-	13	23
			Empididae	-	12	25
			Phoridae-		4	20
			Muscidae	-	9	19
	Crustacea	Isopoda	Asellidae	*Caecidotea*	23	3492
		Amphipoda	Crangonyctidae	*Crangonyx*	19	405
		Decapoda	Cambaridae	-	2	9
	Arachnida	Acari	-	-	16	314
Annelida	Oligochaeta	-	-	-	28	1838
Mollusca	Gastropoda	Pulmonata	Planorbidae	-	14	166
			Physidae	-	13	88
			Lymnaeidae	-	12	75
			Ancylidae	-	2	2
	Bivalvia	Veneroida	Sphaeriidae	-	13	384

Four groups (I – IV) resulted in the greatest sum of IndVal scores, and were chosen to represent distinct invertebrate communities from cluster analysis (Fig. 2.3). Significant indicator taxa are listed below their respected groups in Fig. 2.3. Group I showed the greatest difference among all the groups constructed from cluster analysis and

Figure 2.3 Dendrogram showing clusters formed using Ward's method and a Bray-Curtis distance matrix calculated from log(x + 1) taxon counts. Boxes are drawn around distinct clusters, with indicator taxa listed below each cluster.



had the greatest abundance of the isopod *Caecidotea*. Sites in group I also show the lowest values for taxon richness and values for Shannon diversity and Simpson's dominance (Table 2-3). Sites in group II were distinguished by an abundance of larval Dolichopodidae and aquatic oligochaete worms. This group also contains five of the six ditches that are not within actively farmed fields (two within fallow fields and three within wetland restoration site). The two sites in group III contained the most indicator taxa, including the beetle *Stenelmis* (Coleoptera: Elmidae), five dipteran genera and snails in the family Ancylidae. Sites in group III also had the highest values of taxon richness (Table 2-3). Sites in group IV had the highest abundance of the caddisfly *Ironoquia* (Trichoptera: Limnephilidae), along with two dipteran taxa.

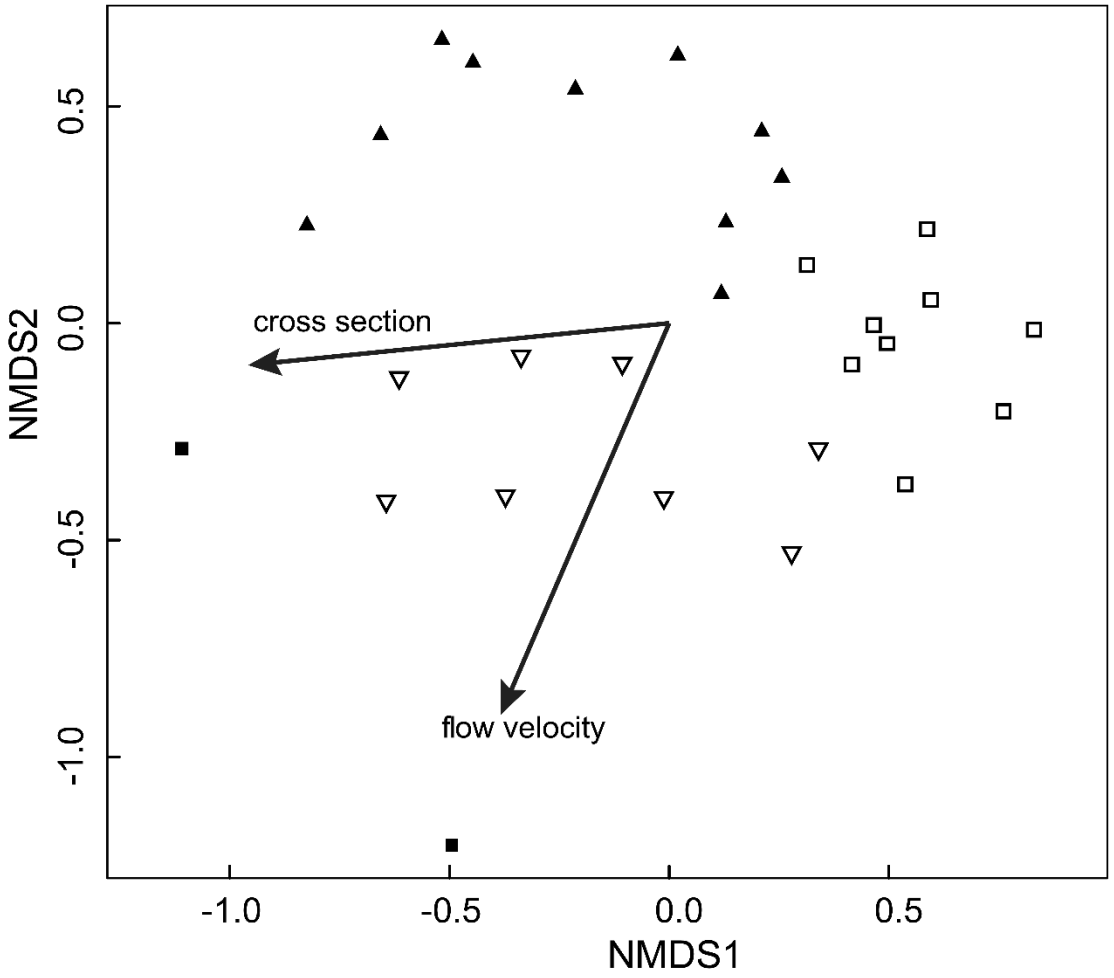
Table 2-3 Community metrics across groups formed by cluster analysis.

	Group Mean ± S.D.			
Community Metric	**I**	**II**	**III**	**IV**
Taxon richness	7.0 ± 2.0	15.2 ± 3.6	28.0 ± 2.8	16.6 ± 6.6
Shannon diversity	0.65 ± 0.27	1.74 ± 0.20	2.46 ± 0.54	1.60 ± 0.62
Organisms/sample	4284 ± 2760	1141 ± 1287	4855 ± 6266	4227 ± 3122
Simpson's dominance	0.32 ± 0.16	0.73 ± 0.06	0.85 ± 0.09	0.63 ± 0.21
% Detritivore	95.47 ± 4.26	82.08 ± 12.20	55.18 ± 7.51	80.32 ± 17.68
% Predator	0.52 ± 0.76	8.67 ± 9.66	18.25 ± 14.24	4.41 ± 4.10
% Scraper	0.00 ± 0.00	0.00 ± 0.00	0.44 ± 0.13	0.00 ± 0.00
% Filterer	0.03 ± 0.10	0.32 ± 0.79	24.60 ± 21.93	10.87 ± 18.97
% Shredder	0.32 ± 0.57	0.56 ± 0.85	0.70 ± 0.50	4.03 ± 5.83
% Herbivore	0.04 ± 0.11	0.10 ± 0.24	0.00 ± 0.00	0.00 ± 0.00

Community – environment relationships

The NMDS ordination produced a stable solution in two dimensions after five runs with a final stress of 17.2 (Fig. 2.4). Overlaying cluster identities onto the NMDS biplot shows that groups II, III, and IV are arranged along axis 2, while there is incomplete separation of group I from groups II and IV along axis 1 (Fig. 2.4). Six environmental variables showed a significant relationship with the NMDS ordination: flow velocity ($r^2 = 0.58$, $P = 0.001$), cross sectional area ($r^2 = 0.56$, $P = 0.001$), ditch depth ($r^2 = 0.52$, $P = 0.001$), redox potential at the sediment surface ($r^2 = 0.29$, $P = 0.012$) and at 2.5 cm ($r^2 = 0.26$, $P = 0.023$), and percent saturation of dissolved oxygen ($r^2 = 0.22$, $P = 0.035$) (Table 2-4). Flow velocity was linearly related to both redox measures and percent saturation of dissolved oxygen, and cross sectional area was linearly related to ditch depth. Flow velocity and cross sectional area were the two environmental variables with the highest r^2 values and were added to the NMDS ordination (Fig. 2.4). The vector for cross sectional area was correlated with the gradient separating group I from groups II, and IV along axis 1. The vector for flow velocity was correlated with the gradient separating groups II, III, and IV along axis 2. Linear regressions were then fit between the two environmental variables (flow velocity and cross sectional area), and FFG diversity. FFG diversity showed a significant positive linear relationship with flow velocity ($r^2 = 0.426$, $P < 0.001$) and cross sectional area ($r^2 = 0.336$, $P = 0.001$).

Figure 2.4 NMDS ordination of sites based on Bray-Curtis distance matrix of log(x + 1) taxon counts. Different points represent individual ditches belonging to the same group based on cluster analysis (open square – group I, closed triangle – group II, closed square – group III, open triangle – group IV). Arrows indicate direction of change in environmental variables fitted to ordination space.



Flow control structures were present in 14 of the 29 ditches sampled (Table 2-1). Presence of flow control structures did not explain any patterns between invertebrate communities of groups of ditches formed by cluster analysis. Group I comprised five ditches with and four ditches without flow control structures. Group II comprised four ditches with and six without flow control structures. Group IV comprised four ditches with and four without flow control structures. Group III comprised two ditches, neither of which had flow control structures.

*Table 2-4 Summary of fitting environmental vectors to NMDS plot. (Significance codes: "***" < 0.001 < "**" < 0.01 < "*" < 0.05).*

Environmental Variable	**r^2**	***P***	
Dissolved oxygen	0.220	0.040	*
Specific conductivity	0.004	0.958	
Water pH	0.142	0.148	
Total solids	0.071	0.403	
Water Total N	0.146	0.134	
Water Total P	0.022	0.799	
Redox 0cm	0.294	0.010	**
Soil pH 0cm	0.035	0.627	
Redox 2.5cm	0.262	0.021	*
Soil pH 2.5cm	0.003	0.963	
Soil Total C	0.110	0.234	
Soil Total N	0.086	0.318	
Bulk density	0.073	0.379	
Plant cover	0.009	0.906	
Detritus cover	0.110	0.203	
Flow velocity	0.584	0.001	***
Cross section	0.562	0.001	***
Ditch depth	0.520	0.001	***
Max. water depth	0.087	0.316	
Flow-control structure	0.070	0.126	

Discussion

Our two main objectives were to investigate patterns of invertebrate community composition among agricultural ditch types and to determine if community composition was related to physical and chemical characteristics of ditches managed to promote improved water quality. We found that the agricultural ditches did not contain homogenous invertebrate communities, and the invertebrate community composition differed between ditch groupings. While invertebrate community composition was related to certain physical and chemical variables managed for water quality, basic physical properties of ditches (flow and size) were primarily responsible for differences in community composition.

The composition of indicator taxa within groups II, IV, and III suggests that ditches varied from forms that are characteristic of long wetlands (temporary lentic habitats) to channelized streams (permanent lotic habitats) (Verdonschot and Higler 1989). Ditches within group II primarily contain taxa that are semi-aquatic, which suggests these sites may have recently dried. Ditches in group IV contain larvae of the caddisfly genus *Ironoquia*, which are strictly aquatic, but are adapted to develop within small, temporary pools and streams (Flint 1958). Ditches in group III have indicator taxa that include *Stenelmis* (Coleoptera: Elmidae) and *Prosimulium* (Diptera: Simuliidae), which are adapted to lotic, erosional habitats (Merritt et al. 2008). Fitting the vector for flow velocity onto the NMDS ordination shows that a gradient in flow velocity underlies the pattern in invertebrate composition. Flow velocity increases from little to no flow in group II to the highest flow in group III.

Sites in group III had greater taxon richness than groups II and IV. This suggests that ditches with flow characteristics of streams may harbor significantly higher diversity than ditches that have no or reduced flow. Sites with greater flow velocities also tended to have greater FFG diversity, which suggests that ditches with flowing water contain niches not present in stagnant ditches. While flow-control structures alter hydrology of ditches and decrease flow rates in order to promote lower redox potentials, we found that flow control structures were distributed among the sites in all taxon groups except group III, which had the highest values for flow velocity. In practice, slowing drainage from a channelized stream to reduce nutrient export could result in the replacement of a community of lotic invertebrates with a lentic community, and subsequently could alter the FFG diversity (McDowell and Naiman 1986). Groups I, II, and IV were all

characterized by species adapted to lentic environments, but we did not find that flow control structures were particularly associated with any one group. Interactions between the effects of flow control structures and other chemical and physical habitat characteristics may have had unique effects on community composition that did not result in one homogenous community across all sites with flow control structures.

Sites in group I had on average a smaller cross sectional area than other ditches, and also supported fewer taxa than other groups. The only indicator taxon for this group was the aquatic isopod genus *Caecidotea*. *Caecidotea* was present in 23 of 29 ditches but had the highest proportional abundances in ditches in group I resulting in low values for the Simpson's dominance metric (Table 2-3). These crustaceans are strictly aquatic, and may be exploiting small ditches as a temporary refuge from predators present in perennial waters (Covich and Thorp 2001). Ditches in group I also had the lowest mean proportional abundance of predatory taxa of all groups (Table 2-3). These small primary drains do not support a large number of species, but may play an important role for species seeking refuge from fish predators in perennial waters (Colvin et al. 2009).

Soil redox potential and dissolved oxygen concentration were the only chemical variables with a significant relationship to invertebrate community structure. Surface soil redox potential may increase as a function of the availability of dissolved oxygen and decrease as a function of the availability of labile organic matter to respiring microbes at this interface (Vepraskas and Faulkner 2001). Therefore, the relationship between redox potential and invertebrate community composition may be mediated by availability of oxygen and organic matter. Most of the invertebrate taxa collected live on or within the benthic substrate (sprawlers and burrowers) and belong to functional feeding groups that

process detrital organic matter (collector/gatherer and shredder). As we sampled exclusively with a D-frame net, we may have underestimated the relative abundance of burrowing organisms. Future studies should explore the role that organic matter processing and bioturbation by this community of invertebrates has on the redox chemistry responsible for nutrient transformations within ditch soils. Finding that none of the other water chemistry variables significantly explained patterns of invertebrate community composition is likely the result of the abundance of taxa adapted to stressful environments found in ditches. The lack of a significant relationship between patterns of invertebrate community composition and nutrient concentrations in either soil or water also suggests that benthic invertebrate communities may not differ between ditches exporting high amounts of nutrients to local watersheds versus those that do not. Therefore, any decrease in nutrient loads will not directly impact any single group of invertebrates, but physical alterations to flow, area, and oxygen concentration related to all aspects of ditch management may alter invertebrate communities.

These results suggest that alterations to water flow in ditches might alter the suitability of ditches as habitat for specific assemblages of aquatic invertebrate species adapted to lotic environments. While not the focus of our study, this result suggests that flow control devices that decrease flow may lower diversity, or alter community composition. We did not find evidence that sites with flow control structures contained a specific level of taxa richness or type of community. If sites in group III however, were to have flow control devices installed and their habitats changed from stream-like to more like a wetland, we would expect a drastic change in community composition and richness. As stated, we cannot rule out the possibility that flow control structures

interacted with other local features of the ditch such as ground water level, area, slope, detritus, soil composition, nutrients, in-channel vegetation, precipitation, or temperature. The effects of flow control structures may also not have manifested themselves in the time period for which sampling was done for this project. Flow control structures may allow water to remain in the ditch longer and delay drying. This could lead to aquatic communities being present in ditches with flow control structures for longer than those without, and communities at these later times may differ than the community of initial colonizers (Welborn et al. 1996, Brooks 2000).

Decisions regarding management of in-stream processes of ditches to improve the quality of water draining agricultural lands may impact habitat quality within ditches. Pollution mitigation strategies that are not implemented in the channel such as grass buffer filter strips adjacent to ditches (Cooper et al. 2004), below-ground biocurtains (Strock et al. 2007), or phosphorus-sorbing soil amendments (Penn et al. 2007, Leader et al. 2008) may be a way to effectively manage ditches for both habitat quality and nutrient pollution. Our results show that flow, ditch size, and chemical measures related to oxygen consumption were the only factors that explained community composition. These characteristics are likely unaffected by activities outside the stream channel. Alterations to flow and ditch area will likely have the greatest impact on community composition and richness, although the greatest impacts are likely to occur when changing stream-like ditches to ditches more characteristics of wetlands. Finding differences in community composition between ditches in this study indicated that maximizing regional diversity of aquatic invertebrates within ditch habitats may depend on maintaining a diversity of physical characteristics of ditches across the agricultural landscape

Chapter 3 - Patterns of burrowing invertebrate communities in small and large agricultural drainage ditches

Abstract

In natural streams, macroinvertebrates play important roles in ecosystem functions, altering rates of transformation and movement of energy and materials, and aquatic macroinvertebrates may have a similar function in agricultural drainage ditches. A quantitative survey of sediment-dwelling macroinvertebrates was done to determine how macroinvertebrate species may alter movements and transformations of agro-chemicals entering drainage ditches. Macroinvertebrates were sampled from sediment cores taken from four pairs of small (field) and large (collection) ditches on Maryland's Eastern Shore monthly from March 2011 to February 2012. 140 taxa were identified and divided into functional groups according to trophic position and modes of burrowing. Patterns in benthic invertebrate density, diversity, and community composition were analyzed in relation to ditch size class and seasonal changes to water quality and quantity. Taxa collected were also compared against macroinvertebrate collections from ditches using D-net sweeps, and against a publically available dataset of stream invertebrates for the region (Maryland Biological Stream Survey 2007-2012) to compare the diversity present in ditches to regional stream biodiversity. Results show that sediment-dwelling invertebrates account for a large proportion (90%) of macroinvertebrates in drainage ditches, but that diversity is lower than natural streams (143 taxa in streams, 78.1 taxa in ditches when richness is rarefied to match stream data abundances). There is no difference in species richness (9.6 taxa/site) or abundance of invertebrates (6,114 ind./m^2)

between small and large ditches, or between seasons. Community composition differs between small and large ditches, but functional group composition does not. Bioturbation by macroinvertebrates is likely the most important process that contributes to ecosystem-scale functions of drainage ditches, including regulation of biogeochemical processes occurring at the sediment-water interface.

Introduction

Headwater streams are important for maintaining water quality at the watershed scale because they are the site of intense biogeochemical transformations that affect transport of materials to all downstream waters (Alexander et al. 2007). Small headwaters are also a significant source of biodiversity of aquatic macroinvertebrates (Clarke et al. 2008, Clarke et al. 2010). These communities of macroinvertebrates have behaviors that serve to modify rates of transformations and transport of materials through the aquatic ecosystem, and therefore can have effects on stream function and water quality (Covich et al. 1999). Changes to the structure of the aquatic macroinvertebrate community can alter the functioning of headwater stream ecosystems (Wallace et al. 1982).

One of the best studied examples of the role aquatic macroinvertebrates play in ecosystem function is how feeding by different functional feeding groups can alter movement of nutrients and organic matter through stream ecosystems (Wallace and Webster 1996). Grazing of periphyton by aquatic invertebrates classified as "scrapers" can alter rates of primary production in streams through removal of algal biomass, and through changes in the periphyton community (Feminella and Hawkins 1995, Holomuzki et al. 2010). "Filterers" are suspension-feeding invertebrates that can alter the rate of transport of organic material by removing suspended matter (seston) from the water

column and depositing feces into the sediment (Malmqvist et al. 2001). The breakdown and transport of allochthonous leaf inputs to streams can be enhanced by feeding of invertebrates classified as “shredders” that break down coarse particulate organic matter to fine particulate organic matter (Crowl et al. 2001).

In densely agricultural areas, extensive drainage networks can completely replace natural headwaters, and are conduits for delivering agricultural pollution to larger streams and rivers (Blann et al. 2009, Dukes and Evans 2006, Carpenter et al. 1998). Drainage ditches are an important component of farming infrastructure for managing soil moisture, and enhance drainage in areas where flat topography and a high water table would otherwise inundate plant roots and cause stress to crops. Ditch networks are typically composed of a system of small field ditches or subsurface tile drains that empty into larger collection ditches that receive water from multiple fields, and then feed into larger streams and rivers (Pavelis 1987). Field ditches are typically ephemeral to intermittent bodies of water, while collection ditches tend to be perennial watercourses that in some cases are mapped and named.

Current research has focused on studying processes occurring within ditches that limit the transport of nutrients and pollutants downstream (Needelman et al. 2007a). The interface between ditch sediments and overlying water is an active site of biogeochemical transformations that can reduce levels of nitrates, phosphates, and pesticides from water draining into ditches (Nguyen and Sukias 2002, Shigaki et al. 2008, Kröger et al. 2008, Cooper et al. 2004, Sharpley et al. 2007, Needelman et al. 2007b). Therefore, there has been much focus on the potential for biogeochemical processes occurring at this interface

to increase the quality of water draining from agricultural lands (Hill and Robinson 2012, Kröger et al. 2013, Usborne et al. 2013).

Besides conveying water from agricultural fields, drainage ditches also provide habitat to many aquatic species, including burrowing species that live within the sediment-water interface (Langheinrich et al. 2004, Leslie et al. 2012, Painter 1999, Simon and Travis 2011). Burrowing animals are ecosystem engineers in soil and sediment habitats, as bioturbation from burrowing activities alters the availability of food resources to below-sediment microbes, changes biogeochemical gradients within sediments, and impacts recruitment of species persisting in dormant stages below the sediment surface (Meysman et al. 2006). To date, studies of ditch sediment characteristics have not taken into account the effect of macrofauna on biogeochemical transformations of nutrients and other chemicals. However, ecosystem-scale effects of bioturbation on nutrient transformations and fluxes have been documented in other aquatic habitats, and may play an important role in ditches as well (Adámek and Maršálek 2012, Usio and Townsend 2004, Lohrer et al. 2004, Chaffin and Kane 2010).

Effects from bioturbation vary among different species, and most of these effects are not well understood and subtle but significant differences may exist between different but related species (Palmer 1997, Mermillod-Blondin et al. 2001). Grouping burrowing species into functional groups allows comparison of the structure of the burrowing community without detailed knowledge of individual species (Lavorel and Garnier 2001). A framework has been proposed for designating these groups which defines functional bioturbation groups based on the physical disturbance created within the sediment, rather than the effect that disturbance has on ecosystem functions (Gerino et al. 2003). By using

this functional group framework, it is possible to infer some of the possible effects of burrowing species diversity on ditch ecosystem function without knowing details of the specific effects of each species.

The overall goal of this study was to quantify sediment-dwelling macroinvertebrate communities in agricultural drainage ditches, and to identify patterns in abundances of different functional groups. Previous work has found that communities of aquatic macroinvertebrates in ditches are dominated by species that live on or in the sediment, possibly as a consequence of reduced habitat complexity in straight, uniform ditches (Leslie et al. 2012). Therefore my collections specifically targeted invertebrates living on or in the sediment to determine what taxa of burrowing aquatic macroinvertebrates are present in drainage ditches, and at what densities they occur. I then assigned taxa collected from this study to different functional groups to determine roles that macroinvertebrates may play in ecosystem functions of ditches. By comparing collections from this study to previous surveys of ditch invertebrates, I determined what proportion of the entire ditch community was associated with the sediment.

Previous work has also shown that macroinvertebrate communities in ditches tend to change as a function of ditch size (Leslie et al. 2012). Drainage ditch networks on Maryland's Eastern Shore are designed such that there are multiple, small ditches contained with a field that all drain into larger, collection ditches that run between multiple fields. Since small (field) ditches tend to hold water intermittently, while large (collection) ditches tend to hold water perennially, my hypothesis was that this difference in habitat permanence would result in differences in the diversity and composition of the invertebrate community between the two classes of ditches and between seasons.

Environmental characteristics were measured to capture differences in conditions between the habitat types, and changes that occur in ditches across seasons. My hypothesis was that different communities associated with each type of ditch are composed of species in different functional feeding groups and functional groups of bioturbation, and may therefore have different effects on ecosystem functions, and show different responses to changes in environmental characteristics across seasons. I compared community and functional group composition of macroinvertebrate communities between field and collection ditches and across seasons to determine whether there are differences between these two habitat types, and whether there are seasonal patterns in the structure and function of the macroinvertebrate community.

Drainage ditch networks have replaced natural headwater streams across many agricultural landscapes and have possibly eliminated the biological diversity and ecosystem functions associated with natural headwaters. I compared aquatic macroinvertebrate communities between natural streams and drainage ditches to determine whether ditches have similar community composition and functional diversity as natural streams. I compared the diversity, community composition, and functional groups of drainage ditches to natural streams in the same area to determine whether drainage ditches support distinct communities of macroinvertebrates, or a subset of the diversity found in natural streams.

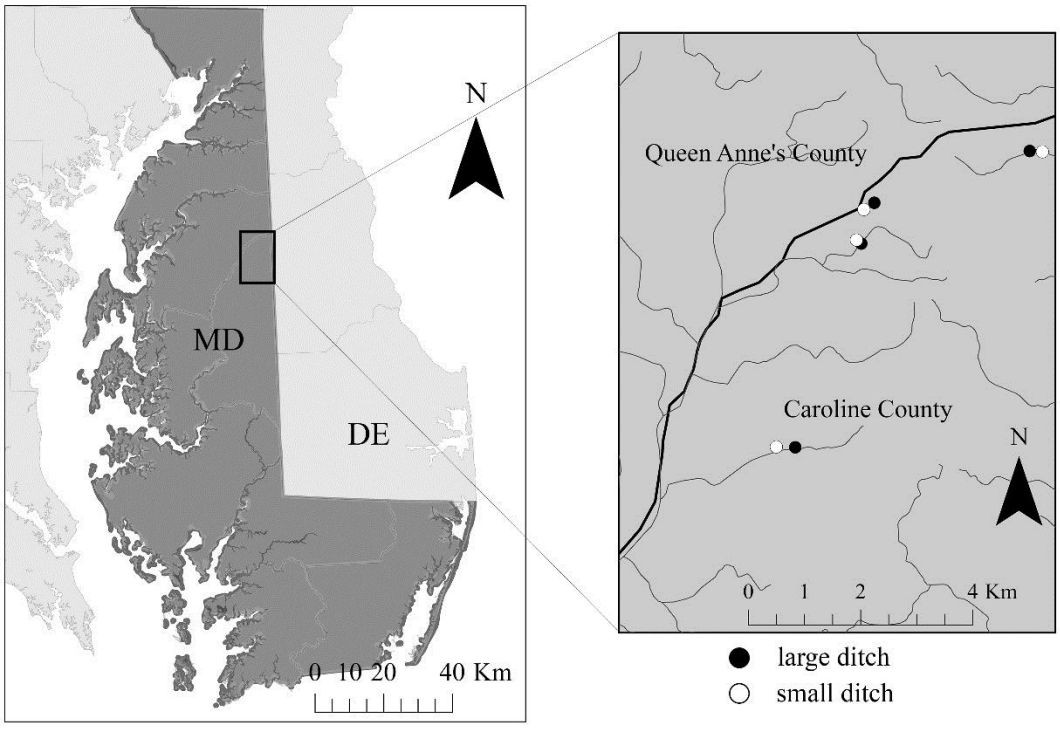
Methods

Site Description

Four farm sites were chosen for the survey on the Eastern Shore of Maryland, within the Choptank watershed in Caroline County (Fig. 3.1). Much of the Eastern Shore is characterized by flat topography and a high water table, which creates naturally poorly

drained soils which could not support field crops without artificial drainage. The drainage networks on Maryland's Eastern Shore are arranged such that multiple, small field drains convey water to larger collections ditches that run between multiple fields and empty into larger streams and rivers. Field ditches are dug and maintained privately by farmers. They tend to be shallow, and as such may only intercept the water table intermittently throughout the year. Collection ditches run between multiple properties, and have been dug and maintained using public funds. Therefore the extent of collection ditches is well documented, and many are mapped as permanent watercourses. These ditches tend to be deeper, and hold water throughout the year.

Figure 3.1 Map of Maryland's Eastern Shore, with inset showing ditch sites in Caroline County.



At each farm, one field ditch and one collection ditch was sampled repeatedly on a monthly basis over the course of a year from March 2011 to February 2012. At each ditch, a 50 m reach was demarcated for repeated sampling. Sampling reaches within

paired field and collection ditches were chosen such that neither was downstream of the other. Sampling was only performed when the ditch contained standing water, which limited collections to aquatic macroinvertebrate species.

Macroinvertebrate Sampling

Quantitative samples of aquatic invertebrates were collected using sediment cores. A steel ring, 18 cm in diameter and 5 cm deep was pushed into the substrate within the ditch and excavated with a shovel to produce a sediment sample of a fixed volume. Three replicate samples were taken from each ditch at each sampling date from random locations along the reach using a random numbers table. Cores were kept in the lab in separate bins with filtered and aerated ditch water to keep macroinvertebrates alive. Cores were rinsed through stacked 4 mm and 0.5 mm sieves to remove fine sediments. Coarse, particulate organic matter (CPOM) was removed, dried, and measured as the mass lost on ignition of material retained within the 4 mm sieve. Macroinvertebrates were removed without magnification, and stored in 80% ethyl alcohol prior to identification to lowest practical taxonomic units, most to genus or species. Taxa of macroinvertebrates collected from sediment cores were compared against collections made with D-net sweeps by Leslie et al. (2012) to determine whether the sampling technique produces an adequate representation of the macroinvertebrate community, or if certain proportions of the total community are systematically excluded by sampling with this method.

Individual taxa were assigned to different functional groups based on their functional feeding group and functional bioturbation group (Gerino et al. 2003). Literature sources were used to determine functional feeding groups and whether individual taxa had burrowing habits (Barbour et al. 1999, Buchanan et al. 2011, Merritt

et al. 2008, Millard et al. 2001, Stribling et al. 1998). When multiple feeding groups were listed for a single taxon, the primary designation was used. Burrowing modes were one of three categories, which reflect the physical change that organisms produce to the sediment: biodiffusors randomly scatter surficial sediments, conveyors feed at depth and deposit sediment at the surface, and gallery diffusors build networks of burrows that are actively irrigated (Gerino et al. 2003). Taxa listed as having a sprawling habit are associated with the sediment surface, but do not necessarily excavate defined burrows, and were placed in the biodiffusor category (i.e. the isopod, *Caecidotea*). Deposit-feeding taxa with a burrowing habit were assigned to the conveyor category (i.e. the oligochaete worm, *Limnodrilus hoffmeisteri*). Burrowing taxa that feed within their burrows (i.e. collector-gatherer or filter feeder) were assigned to the gallery diffusor category (i.e. larvae of the midge, *Chironomus*). Burrowing taxa that do not feed within their burrows (i.e. predators or shredders) were assigned to the biodiffusor category (i.e. larvae of the horse fly, *Tabanus*).

The Maryland Biological Stream Survey (MBSS) has been an ongoing project monitoring stream health across the state of Maryland as a collaborative effort between different state, local, and private agencies. Data included in this document were provided by the Maryland Department of Natural Resources Monitoring and Non-tidal Assessment Division. Comparing the macroinvertebrate communities of drainage ditches to the natural streams can give insight into the value of drainage ditches as a source of biodiversity to the area. The MBSS uses aquatic D-net sweeps (0.6mm mesh) allocated proportionately to representative habitat types to collect aquatic invertebrates. These collections are subsampled to 100-individual counts before being identified to genus.

Although direct statistical comparisons are limited due to the difference in the nature of the two datasets, comparisons of the benthic invertebrate community of ditches to MBSS data from streams can give insight into the proportion of the regional species pool represented in drainage ditches. To make the two datasets more comparable, species richness values from ditch surveys were calculated after rarefaction of ditch communities to a size comparable to the MBSS data. To compare community composition the taxonomies of the two datasets had to be adjusted. First, a genus-level dataset was created by combining species of the same genus to a single taxon and eliminating taxa from higher taxonomic categories (i.e. family or order-level identifications). Second, a family-level dataset was created as a more conservative estimate of the difference in community composition. Finally, both datasets were transformed to presence/absence data to account for differences in abundance between the two sampling techniques. All statistical analyses were performed on all versions of the community dataset.

Environmental Measures

Physical and chemical measures were made at each ditch to document seasonal changes in physical and chemical conditions within each ditch. Specific conductivity and temperature were measured at a single downstream point using a handheld meter (YSI 30, YSI inc., Ohio, USA). A grab sample was also taken from this point and returned on ice to the laboratory to measure pH (Corning 340, Corning, New York, USA). Wetted width, maximum depth, and flow velocity (Flow Mate Model 200, Marsh McBirney inc., Colorado, USA) were measured at six equally spaced points along the sampling reach. To gain a measure of the variability among the environmental measures at each site, coefficients of variation were calculated for width, depth, and flow velocity.

Data Analyses

Differences in measured environmental parameters, as well as mean abundance, taxon richness, and Shannon diversity between field and collection ditches across seasons were determined using repeated-measures ANOVA, using ditch size and season as fixed factors. For these calculations, replicate macroinvertebrate samples were combined to give a single value for each sample date. Macroinvertebrate community and environmental data were pooled across months to give seasonal values (Mar-May=spring, Jun-Aug=summer, Sep-Nov=fall, Dec-Feb=winter). Means were calculated for environmental and macroinvertebrate data to account for missing samples from small ditches during dry periods.

Dufrene-Legendre indicator species analysis (IndVal) was used to determine which taxa tend to be significantly associated with either field or collection ditches (Dufrene and Legendre 1997). For this analysis, replicate samples were combined to give a single value for each sample date. This analysis uses the frequency and abundance of taxa in each type of ditch to determine whether the presence and abundance of a species is significantly greater in one type of habitat than the other.

Multivariate ordination was used to visualize whether small and large ditches have distinct burrowing communities and whether those communities show any changes across seasons. Multivariate ordinations were performed on both the table of taxa by site and the table of functional groups by site. Taxa that were found only from a single sample were removed for community composition analyses. Non-metric multi-dimensional scaling was used to determine patterns in the composition of macroinvertebrate taxa and functional groups. If field and collection ditches harbor

distinct communities, then sites from those two size classes should form separate clusters in ordination space. Likewise, if communities change as a function of season, then sites should cluster in ordination space according to season. Permutational MANOVA was used to determine whether there were significant differences in community and functional composition between small and large ditches and across seasons. The four farm sites were used as a grouping variable within which permutations were contrained. Non-metric multidimensional scaling was also used to determine patterns in community and functional group composition between ditch communities and communities from local streams. Permutational MANOVA was used to determine whether the community composition of ditches is different from streams, and whether functional group composition of ditches is different than streams.

All statistical analyses were performed using the statistical program R 3.1.0 (R Core Team 2014). Linear mixed-effects models were written using the package *nlme* (Pinheiro et al. 2014), and ANOVAs were performed using the *car* package (Fox and Weisberg 2011). Matrices of contrast coefficients were built using the package *contrast* (Kuhn et al. 2013), and multiple means comparisons were performed using the package *multcomp* (Hothorn et al. 2008). Indicator value analysis was performed using the package *labdsv* (Roberts 2013). Non-metric multidimensional scaling, permutational MANOVA, and rarefaction were performed using the package *vegan* (Oksanen et al. 2013). Graphics were produced using the package *ggplot2* (Wickham 2009).

Results

Collection ditches retained water throughout the year, allowing continuous sampling, while all field ditches were dry for at least one month during the summer. Two

field ditches (LMsm and EDsm) were dry from sampling dates in May through July, one (BTsm) was dry from June through July, and the fourth field ditch (BVRsm) was only dry for the July sample date (Table 3-1). All field ditches were re-wetted for the August sample date following heavy precipitation from a hurricane that passed through the area. There was a significant effect of season (χ^2=275.76, df=3, P<<0.001) and no effect of ditch size on water temperature. Seasonal changes in water temperature follow the usual pattern for Maryland air temperatures, with the highest temperatures recorded in the

Table 3-1 Means of environmental variables measured across ditches. Values for months where ditches were dry were omitted from mean calculations. Depth and width measurements represent dimensions of water contained within the ditch, and not overall size of the ditch. C.V. = coefficient of variation of multiple measured environmental variables. Site abbreviations ending in "sm" are field ditches, and site abbreviations ending in "lg" are collection ditches.

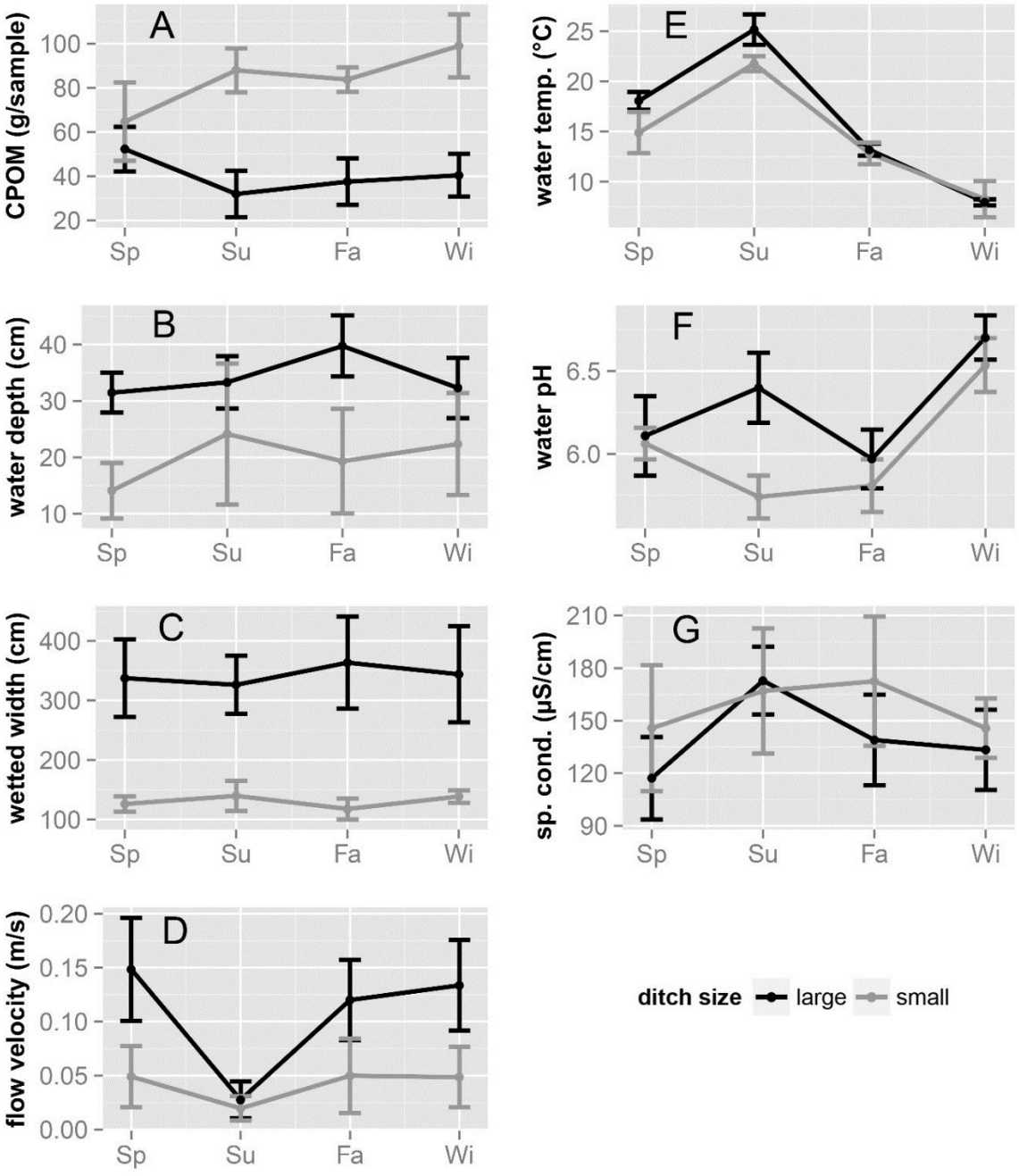
Ditch	pH	Sp. Cond. µS cm^{-1}	Depth cm	C.V. %	Width cm	C.V. %	Flow m s^{-1}	C.V. %	Dry month
BTsm	5.96	100.0	17.3	26	127.7	15	0.08	40	2
BVRsm	5.79	134.6	9.1	31	119.3	52	0.10	61	1
EDsm	6.42	221.6	44.5	8	165.5	11	0.00	0	3
LMsm	6.22	168.7	7.4	74	107.8	57	0.00	0	3
BTlg	5.81	91.1	32.1	17	388.7	15	0.10	46	0
BVRlg	6.45	130.9	25.3	15	305.6	21	0.20	32	0
EDlg	6.41	193.4	32.9	30	181.7	21	0.10	76	0
LMlg	6.51	147.0	46.5	10	495.0	6	0.03	37	0

summer, and the lowest in the winter (Fig. 3.2e). Two collection ditches (LMlg and BTlg) experienced temperature changes of 20.2°C between the summer and winter months, while the lowest temperature change of 9.5°C was recorded from a field ditch (BVRsm). There was a significant effect of season on ditch pH (χ^2=62.31, df=3, P<<0.001) and mean flow velocity (χ^2=30.54 df=3, P<<0.001) with no effect of ditch size, but a significant interaction between season and size. Within small ditches, pH was slightly higher in winter than other seasons, and in large ditches, pH was lower in the fall

than other seasons (Fig. 3.2f). Across all sites and seasons, the range in pH values was 5.4 – 6.9. Flow velocities were significantly lower in large ditches in the summer, while there were no differences in flow velocity among small ditches between seasons (Fig. 3.2d). There was no effect of season or ditch size on specific conductivity (Fig. 3.2g), mean water depth (Fig. 3.2b), or coefficients of variability for flow velocity, mean width, and mean depth. There was a significant effect of ditch size on mean wetted width (χ^2=9.82, df=1, *P*=0.002), with large ditches being wider than small ditches across all seasons (Fig 3.1c). Ditch size had a significant effect on CPOM collected with sediment cores (χ^2=8.73, df=1, *P*=0.003), but there was also a significant interaction with season (χ^2=19.73, df=3, *P*<0.001). Pairwise comparison of ditch sizes across seasons shows that small ditches have significantly more CPOM than large ditches during every season except spring (Fig. 3.2a).

A total of 261 individual sediment cores were collected, yielding 140 macroinvertebrate taxa, spanning six phyla. After determining habits from published sources, 101 taxa were identified as burrowing species, with a total of 39,431 individuals. Most burrowing taxa (65) were within the Class Insecta, among which 49 taxa were in the order Diptera, and of those, 32 were genera of the family Chironomidae. Only 36 non-insect taxa were collected, but their abundance represented a majority of individuals collected (72.8%). The majority of the community (90%) was dominated by the 11 most abundant taxa (Table 3-2). Repeated measures ANOVA showed that taxon richness, individual abundance, and Shannon diversity of burrowing taxa did not differ between field and collection ditches or between seasons (Fig. 3.3). With no significant differences in taxon richness or invertebrate abundance between ditch size classes or across seasons,

Figure 3.2 Summary of environmental variables measured over seasons for small and large ditches. Error bars represent +/- 1 SEM. Seasons are abbreviated: "Sp"-Spring, "Su"-Summer, "Fa"-Fall, "Wi"-Winter.



the overall mean taxon richness by season was 9.6 (range 3-18) and the overall mean abundance per ditch was 6,114 ind./m^2 (range 170-44,620 ind./m^2). Without averaging over months within a season, the overall mean taxon richness by month increases to 16.9 (range 3-33).

Comparison of the taxa collected from sediment core samples to a survey of agricultural drainage ditches on the Eastern Shore using D-net sweeps (Leslie et al. 2012) and to collections from local streams (MBSS) shows similar taxa collected from the three sources. Differences in the taxonomy of oligochaete worms caused large discrepancies in taxon richness and abundance between the three datasets, so results are presented for the

Table 3-2 Top 11 most abundant macroinvertebrate taxa, which comprise 90% of the entire ditch benthic community. Asterisk (*) represents significant association of taxon with size class of ditch (α=0.05). Abundance measures are the mean density of individuals of that taxon collected across all samples from each size class of ditch. IndVal represents the statistic calculated from indicator species analysis for large and small ditches, and a P-value showing the significance of the association of taxa with one size class over the other.

	Abundance (ind./m^2)		**IndVal**		
Taxon	**Large**	**Small**	**Large**	**Small**	***P***
Haplotaxida (aquatic worms)					
Limnodrilus hoffmeisteri	2,062	1,497	0.56	0.42	0.341
Ilyodrilus templetoni	1,274*	31	0.89	0.01	0.001
Spirosperma nikolskyi	2	456*	0.00	0.51	0.001
Dero digitata	97	41	0.31	0.08	0.197
Isochaetides curvisetosus	65	61	0.18	0.12	0.631
Veneroida (clams)					
Pisidium	168	430*	0.17	0.53	0.016
Musculium	293*	8	0.37	0.00	0.006
Isopoda (aquatic sow bugs)					
Caecidotea	106	1,304*	0.04	0.71	0.001
Amphipoda (scuds)					
Crangonyx	9	241*	0.01	0.86	0.001
Diptera (fly larvae)					
Chironomus	2,157*	30	0.58	0.01	0.017
Cricotopus/Orthocladius	70	91	0.14	0.09	0.849

Figure 3.3 Summary of community metrics measured across seasons for small and large ditches. Error bars represent +/- 1 SEM. Season abbreviations are: "Sp"-Spring, "Su"-Summer, "Fa"-Fall, "Wi"-Winter.

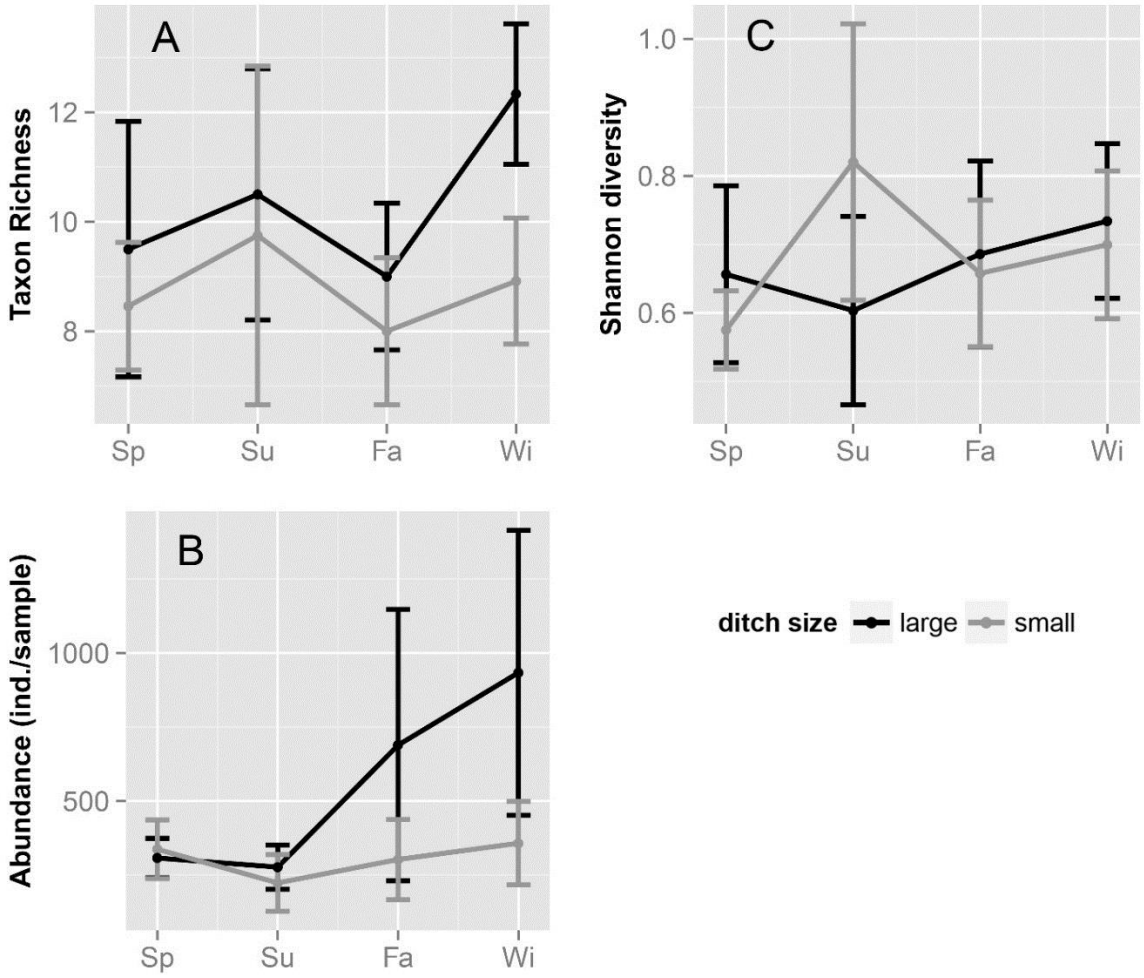
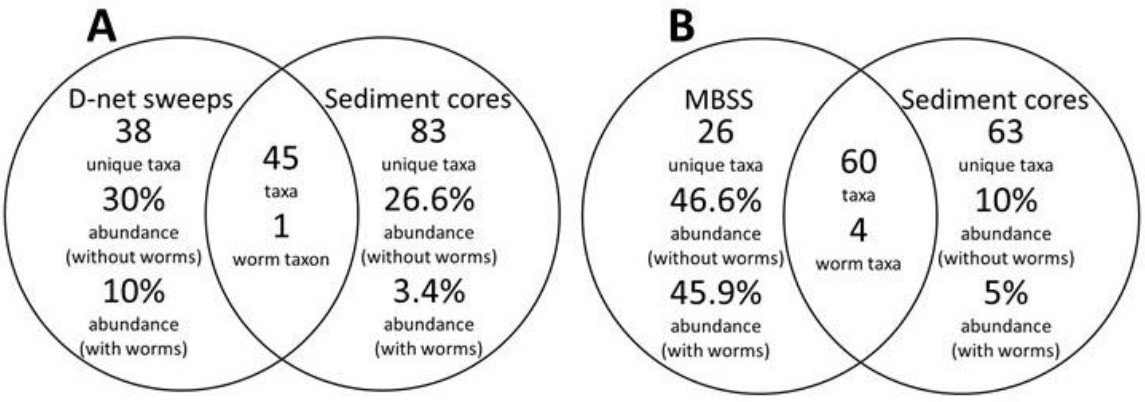


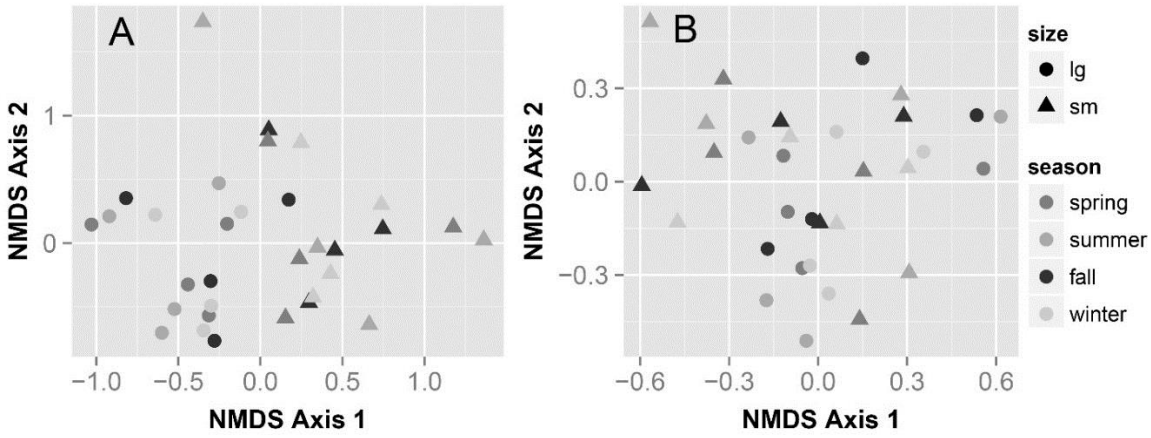
Figure 3.4 Venn diagrams showing shared and unique taxa between collections from ditch sediment cores and both ditch D-net sweeps (A) and collections from local streams (MBSS) (B). Shared taxa and abundances of unique taxa are displayed with and without counting oligochaete worms.



invertebrate communities first excluding oligochaete taxa, and then grouping them at the class level for comparisons between the two ditch samples, and at the family level for comparison between ditch sediment cores and local stream samples. Collections from sediment core samples included 45 of the same taxa collected from D-net sweeps of ditches, which accounts for a total individual abundance of 70% from D-net sweeps and 74.4% from sediment cores (Fig. 3.4a). When oligochaete taxa are pooled and added to the calculation of percent shared abundances, D-net sweeps of ditches share 90% and sediment cores share 96.6% of their total abundance. Ditch sediment cores share 60 taxa in common with collections made from local streams, which accounts for 90% of the total abundance in ditch samples, and 53.4% of the total abundance in stream samples (Fig. 3.4b). When oligochaete taxa are included in the calculations, total abundance of shared taxa increases to 95% for ditch sediment core samples and 54.1% for stream samples.

Single-table ordination of the burrowing invertebrate community shows that large and small ditches tend to separate from one another in ordination space (Fig. 3.5a). The site-by-taxa data table was Wisconsin square-root transformed before calculating the Bray-Curtis distance matrix. Using a random start configuration, a stable solution was found in two dimensions with a stress of 0.17. Permutational MANOVA shows that there is a significant difference in macroinvertebrate community composition between small and large ditches (F=3.622; df=1, 27; *P*=0.001), but there is no effect of season on community composition (F=0.682; df=3, 27; *P*=0.644). Results of the IndVal analysis showed 20 taxa are significantly associated with collection ditches, and 12 taxa associated with field ditches. Table 3-2 shows associations of the top 11 most abundant taxa with the two classes of ditches. Three of the top 11 most abundant taxa are significantly associated with collection ditches, while four taxa are significantly associated with field ditches. Four of the dominant taxa were equally associated with both class of ditch, including the most abundant species (*Limnodrilus hoffmeisteri*).

Figure 3.5 NMDS biplot of site by species dataset (A) and the functional group by site dataset (B). Points represent site scores. Small (triangles) and large (circles) tend to occupy different space within the biplot A but not biplot B, indicating different taxonomic composition but similar functional groups between ditch sizes. Seasons (shades of gray) do no tend to aggregate in any pattern, indicating no effect of seasonality on taxonomic or functional group composition.



After assigning taxa from the community dataset to functional groups, the site-by-taxa table of 140 taxa becomes distilled to ten functional groups for feeding and burrowing. The ditch benthic community is dominated by taxa in the collector-gatherer functional feeding group (83.6%), followed by collector-filterers (7.9%), predators (3.8%), shredders (3.2%), scrapers (1.5%), and parasites (<0.1%). Nearly all (97.1%) of the benthic community had burrowing habits, which were divided into conveyors (49.5%), gallery-diffusors (29.6%), and biodiffusors (18.0%). Single-table ordination of functional groups does not reveal discrete grouping based on size or season (Fig 3.5b). The site-by-functional group table was also Wisconsin square-root transformed before calculating the Bray-Curtis distance matrix. Using a random starting configuration, a stable solution was reached in two dimensions with a stress of 0.14. Permutational MANOVA found no significant differences in functional group composition between small and large ditches ($F=1.100$; df=1, 27; $P=0.199$), and no difference in functional group composition between seasons ($F=0.833$; df=3, 27; $P=0.373$).

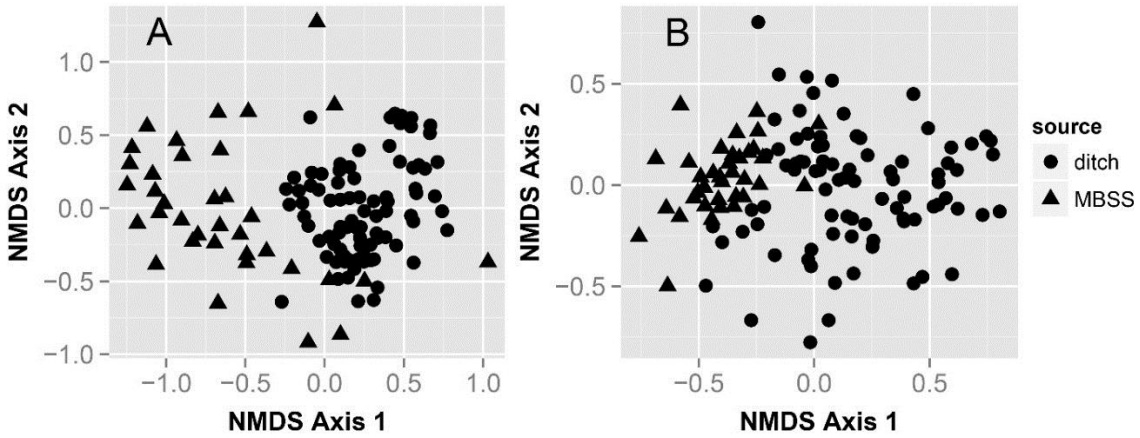
Results of the MBSS for Caroline County from 2007 to 2012 found 206 taxa of macroinvertebrates from 36 samples taken from 24 sites in four sub-watersheds of the Choptank and Nanticoke Rivers. To make the dataset comparable to the community data for ditch macroinvertebrates, 30 taxa (higher than genus level) were deleted to prevent inflation of diversity calculations, which eliminated 209 individuals and reduced the dataset to 176 taxa. Mean taxon richness per site was 23 (range 6-36), and the total of individuals represented in this dataset was 3,708, with a mean of 103 individuals per sample. The communities of invertebrates in the MBSS dataset were composed mostly of insects, with 143 taxa representing 77.8% of the total abundance. All congeneric species

from the ditch community dataset were combined to produce a dataset of genus-level data comparable to the MBSS data, which resulted in nine species being reduced to three genera for a net loss of six taxa. Rarefaction of ditch samples collected from sediment cores to estimates based on 103-individual subsamples gives a mean richness of 11.9 taxa per ditch. Rarefaction of the total pool of benthic ditch invertebrates to a total of 3,708 individuals gives a rarefied estimate of 78.1 taxa in the ditch regional species pool.

Single-table ordination of the combined ditch and MBSS datasets shows that streams and ditches have different community composition (Fig. 3.6a). Non-metric multidimensional scaling of every iteration of the combined dataset shows separation of the two types of habitat in ordination space. Therefore, results of only the most conservative version of the combined dataset are presented. Five taxa of higher level than family were deleted from the dataset (Acari, Amphipoda, Diptera, Lepidoptera, and Nematoda), which excluded 39 individuals from the analysis. The family-by-site data table of presence/absence data was used to calculate a Bray-Curtis distance matrix. A stable solution was found in two dimensions resulting in a stress of 0.21, but clearly showing separation between the two habitat types. Permutational MANOVA shows that community composition between the two habitat types is significantly different ($F=30.123$; $df=1, 121$; $P=0.001$). Non-metric multidimensional scaling of functional groups of ditch and MBSS data shows that streams and ditches segregating in ordination space (Fig. 3.6b). Permutational MANOVA shows that the difference in functional group composition between ditches and streams is significant ($F=35.827$; $df=1, 121$; $P=0.001$). The functional feeding group composition of MBSS streams is more even than ditches, with collector-gatherers representing 33% of the community, followed by filterers (27%),

predators (16%), shredders (13%), and scrapers (12%). The majority of the MBSS community were non-burrowing taxa (54%), with the majority of the remainder represented by biodiffusors (40%), and a minority of gallery-diffusors (5%) and conveyors (2%).

Figure 3.6 Non Metric Multi-Dimensional Scaling biplots of community and functional group composition of drainage ditches and streams surveyed by the MBSS. Sites are represented by circles for ditch sites and triangles for MBSS sites. (A) Taxonomic and (B) functional group composition of the two habitat types are distinct, which is represented by their clustering in ordination space.



Discussion

Small, headwater streams are important not only for supporting biodiversity not found in larger downstream habitats, but also as locations of intensive biogeochemical cycling of nutrients and carbon, which can determine the fate and delivery of energy and nutrients to the rest of the watershed (Peterson et al. 2001, Alexander et al. 2007). Macroinvertebrates can have ecosystem level effects on rates of transformations and transport of organic matter and nutrients within the aquatic ecosystem. One way that macroinvertebrates can impact ecosystem functions is through their feeding, whereby they both physically and chemically change the organic matter they consume (Wallace and Webster 1996, Wallace et al. 1982, Cummins and Klug 1979). Another way they can

impact the ecosystem is through bioturbation, or the physical disruption of aquatic sediments, which can alter the exchange of materials between the sediment and surface water (Meysman et al. 2006). The objective of this study was to determine what burrowing macroinvertebrate species inhabit small and large agricultural drainage ditches on Maryland's Eastern Shore, and to determine the potential roles of these communities in ecosystem functions of ditches.

There have been several other studies from other parts of the world that have estimated species richness of drainage ditches using different techniques. Ranges of local species richness in ditches are from 13 (Davies et al. 2008, Williams et al. 2003) to 75 (Verdonschot et al. 2011), and estimates of landscape scale richness range from 46 (Simon and Travis 2011) to 360 (Verdonschot and Higler 1989). These studies make use of hand-held nets (mesh 0.5-1mm) to sample invertebrates, and so communities include species associated with the sediment as well as the water column and vegetation. Some of the variation in richness estimates is due to differences in taxonomic resolution in the invertebrate dataset. For example, oligochaete worms and chironomid midge larvae are often not identified past the level of class or family, respectively (i.e. Davies et al. 2008, Leslie et al. 2012). Taxon richness can increase as a function of taxonomic resolution for these groups, as Verdonshot et al. (2011) found 10 taxa of oligochaetes and 43 taxa of chironomids, and Verdonschot (1987) found 28 species of oligochaetes in drainage ditches, including five species also found in this study. The purpose of this study was to specifically quantify the community of burrowing invertebrates in drainage ditches, and as a result only sampled species of invertebrates living on or in the sediment, and so is only representative of a subset of the entire aquatic community within ditches. The

sediment-dwelling community nevertheless constitutes a significant fraction of the total community, with a mean monthly alpha richness of 16.9 species these estimates from sediment cores are as high as or higher than richness values obtained from sweeping with aquatic nets. The shared taxa between sediment cores and D-net sweeps also accounts for over 90% of the total abundance of individuals collected, and unique taxa collected by the two collection methods occur in low abundances. A substantial amount of diversity in drainage ditches lives below the surface of the sediment, so studies of total drainage ditch biodiversity will likely have to use multiple sampling techniques to sample the entire community effectively. Examples of some taxa that may not have been collected using aquatic nets include the aquatic larvae of the weevil *Lissorhoptrus oryzophilus*, the freshwater mussel *Elliptio fisheriana*, and two stygobiont taxa: *Stygonectes* and *Haplotaxis gordeoides*. The weevil larva feeds on the roots of rice cutgrass (*Leersia oryzoides*), and the freshwater mussel and the stygobiont species are capable of burrowing deep within sediments, and therefore may not be sampled effectively using nets.

Comparison of rarefied species richness of benthic invertebrates in ditches with species richness in streams measured by the MBSS shows that ditches have much lower diversity than natural streams at local and landscape scales. This result is consistent with findings from comparative studies between ditches and other natural bodies of water in Europe, where ditches there were found to have the lowest diversity at the local scale of any habitat types sampled (Davies et al. 2008, Williams et al. 2003). Although the extent of comparisons that can be made with these two data sets is limited, drainage ditches clearly have lower macroinvertebrate diversity than natural streams in the same region.

Collections from ditch sediment cores contain a majority of the taxa collected from natural streams. These shared taxa account for 95% of the abundance in drainage ditches, but only 54% of the total abundance in streams. This suggests that ditches support predominantly generalist aquatic taxa that are capable of surviving harsh conditions within ditches, but are also common in other aquatic habitats. Many of the taxa found in ditches but not streams are taxa that are typically found in lentic habitats, such as nymphs of libellulid dragonflies, glossiphoniid leeches, and dytiscid beetles, suggesting that drainage ditches support a mixture of both stream and pond/wetland species.

There were no differences between large and small ditches for most environmental variables measured during this study except wetted width, CPOM, and whether or not the ditches were dry over the summer. Therefore differences between benthic communities of large and small ditches could not be explained by differences in water quality or flow velocity, and instead must be due to either differences in hydroperiod or in size. Large ditches did not undergo drying during the summer, and were not subject to changes in the community as a result of colonization and succession of species. Large ditches have a more stable habitat and a more continuous community, while small ditches are subject to changes in community composition as a result of the aquatic habitat disappearing. Species that persist in large ditches must outcompete other species and avoid predators for a place within this relatively stable habitat (Wellborn et al. 1996). For species to persist in small ditches, they must recolonize either from desiccation resistant stages, or by recolonization from adjacent habitats. Therefore I would expect the differences in community composition between small and large ditches to also reflect differences in traits associated with survival under these two conditions. Of

the top 11 most abundance taxa, there were four that were significantly associated with small ditches (*Crangonyx sp., Pisidium sp., Spirosperma nikolskyi,* and *Caecidotea sp.*). *Crangonyx* and *Caecidotea* are both genera of aquatic crustaceans (orders Amphipoda and Isopoda, respectively). There are species of both genera that are capable of living in shallow aquifers, but the species collected from this study did not show characteristics of subterranean lifestyle. Instead, these two genera are fairly mobile within surface waters, and possibly colonize small ditches as they re-wet from adjacent permanent bodies of water. *Pisidium* is a genus of aquatic clam that is capable of surviving brief periods of desiccation by retaining its offspring internally within a marsupium (Smith 2001). Little information is available on the biology and ecology of the oligochaete worm *S. nikolskyi*, however this species likely survives periods of desiccation by retreating deeper within the hyporheic zone to find moist refuges.

Differences in water permanence in ditches may also result in differences in the physical structure of the benthic habitat, and may change the suitability of the ditch as habitat for different species. Vaughan (2008) described differences in the sediment profiles of field and collection ditches from Maryland's Eastern Shore, and documented the development of pedogenic structure in small ditches as a result of wet and dry cycles while larger ditches tended to have structureless, single-grained sediment. The sediment structure of smaller ditches may produce larger interstices that can be utilized by benthic invertebrates searching for refuges. The presence of rooted vegetation may also play a role in determining the composition of benthic invertebrates in ditches. Field ditches sampled in this study all supported rooted vegetation that was able to establish in the channel during dry periods, while collection ditches only supported rooted vegetation

along the banks. Vegetation growing within the channel is likely the reason small ditches tended to have higher CPOM levels than large ditches. The presence of plant roots in field ditches may provide different physical habitat as well as a food resource to burrowing invertebrates that is not available in collection ditches. Although there were no consistent differences in flow velocities between small and large ditches, differences in flow characteristics may further shape the sediment habitat. Two of the field ditches measured during this study never had measurable flow, while all of the collection ditches had measurable flow velocities during most of the year. Higher flow velocities may increase the transport of fine sediment particles, leaving coarse-textured sediment, while ditches with little or no flow may have increased deposition of fine sediment particles (Allan and Castillo 2007). Future studies would be needed to separate the effects of differences in mineral particle size distribution and the development of higher-level sediment structure on the benthic community composition of drainage ditches.

The communities of benthic invertebrates collected from these ditches are representative of streams with impaired function. There is very little in the way of functional feeding diversity, with most species representing detritivorous collector-gatherer feeding modes. Communities are numerically dominated by individuals that are non-insect taxa such as oligochaetes and crustaceans, and the most abundant insects are larvae of chironomid midges, which can be indicative of organic pollution. The most obvious impairments to the ditches would be loss of riparian habitats and nutrient enrichment from adjacent agricultural fields, followed by channel modification. Vegetation adjacent to ditches was composed primarily of grasses and weedy vines, which may reduce bank erosion, and are typically mowed every fall. Without effective

shading from riparian vegetation, ditches are subject to wide annual fluctuations in temperature. Measured water temperature ranges for ditches in this study were 0.6-32.2°C for the year, which excludes any taxa that are sensitive to temperature changes from inhabiting ditches. Although not measured in this study, high temperatures experienced in summer months likely also reduce the amount of dissolved oxygen available to invertebrates. Straight channel designs and periodic dredging prevent the development of heterogeneity in channel morphology, which may prevent suitable microhabitats, such as riffles and pools, from developing in ditches. These abiotic stresses prevent the establishment of a community with more diversity in functional feeding groups, which may have consequences on rates of ecosystem functions, such as the breakdown and transport of organic matter.

Bioturbation by benthic invertebrates may be the most important function of the invertebrate community in drainage ditches, with conveyors, gallery-diffusors, and biodiffusors being well represented in the community. Many studies have shown how bioturbation can alter physical and chemical properties of sediment and redistribute materials between the sediment and overlying water, and these changes may have important consequences for drainage ditches. Physical disturbance of sediment by biodiffusors and pelletization of sediment by conveyors can alter rates of sediment accrual and erosion in aquatic habitats (Usio and Townsend 2004, Montserrat et al. 2008). Irrigation of burrows by gallery-diffusors can increase rates of solute transport between sediment porewater and surface water (Chaffin and Kane 2010). Burrows can also increase the availability of dissolved oxygen in anoxic sediments, which can alter rates of oxidation-reduction reactions, including the cycling of nitrogen compounds, and

the stability of iron oxides and oxyhydroxides that can bind phosphate (Lewandowski et al. 2007, Mermillod-Blondin et al. 2001, Kristensen 1988). The availability of oxygen to subsurface sediments can also stimulate microbial production, which in turn can further alter rates of biogeochemical transformations (Webb and Eyre 2004, van de Bund et al. 1994). Interactions between all of these effects can lead to increased ecosystem function, as physical, chemical, and biological heterogeneity of the sediment are increased (Lohrer et al. 2004). However, the specific effects of burrowers on ecosystem function may depend upon the habitat context in which the bioturbation is taking place (Mermillod-Blondin and Rosenberg 2006).

Maximizing biogeochemical and physical processes to reduce the delivery of agricultural pollution to receiving waters has become an active area of research for drainage ditches. Through manipulations of ditch morphology and regulation of drainage rates, drainage ditches have become a valuable tool for reducing the transport of sediment and nutrients to streams and rivers. Some studies have documented the effects of emergent plant communities (Cooper et al. 2004) and microbial communities (Shigaki et al. 2008) on water quality, but currently there have been no studies that take into account the role of the benthic invertebrate community. Macroinvertebrate communities within drainage ditches may have an effect on rates of nutrient transformations and transport, as well as sediment deposition within ditches as a result of bioturbation. In many areas, drainage ditches are the initial site where nutrients and pesticides enter the watershed from agricultural fields. Bioturbation by burrowing invertebrates living within drainage ditches could alter rates of biogeochemical processes that affect whether these chemicals

remain bound within the sediment, or become released to the water column and transported downstream.

Conclusions

The overall goals of this study were to determine the structure and putative function of the macroinvertebrate community in drainage ditches, and to determine how they vary over size classes of ditches and between seasons. Drainage ditches support communities of benthic macroinvertebrates that may play roles in ecosystem functions. The structure of these communities differs greatly from what is expected for headwater streams of the coastal plain, and therefore the functions of those communities differ as well. Diversity among functional feeding groups is missing in ditch environments, but there is a great diversity of burrowing species representing different functional bioturbation groups. The most important function of these macroinvertebrate communities is likely bioturbation of the sediment, which could have effects on physical and biogeochemical processes controlling the transformation and transport of agricultural pollution. Community composition did not change across seasons. The structure of the macroinvertebrate community differed between small and large ditches as a result of small ditches drying in the summer, however functional group composition is similar in both size classes. Future studies of the function of macroinvertebrates in drainage ditches should focus on effects of different bioturbation functional groups on nutrient cycling and other biogeochemical processes occurring across the sediment-water interface.

Chapter 4 - Burrowing macroinvertebrates alter phosphorus dynamics in drainage ditch sediments

Abstract

Studies of macroinvertebrate communities in drainage ditches have focused on documenting the biodiversity from natural streams and wetlands that is supported by these human-altered environments, but none have explored the ecosystem functions provided by those biological communities in ditches. Bioturbation by burrowing benthic invertebrates in ditch sediments may change rates of biogeochemical processes controlling fluxes of nutrients across the sediment-water interface. Here, I used microcosms to test the effect of four different species of burrowing invertebrates (*Ilyodrilus templetoni, Limnodrilus hoffmeisteri, Crangonyx* sp., *Chironomus decorus* S.G.), representing three different functional groups of bioturbation, on exchanges of phosphorus between sediment and water from a drainage ditch. These effects were measured across a range of background sediment and water characteristics, representing variability within ditches. Results show that all four species reduced concentrations of soluble reactive phosphorus (SRP) in the surface water relative to controls under some environmental conditions, but two species (*L. hoffmeisteri* and *C. decorus*) also increased SRP concentrations under some environmental conditions. Decreases in phosphorus concentration were linked to changes in the sediment redox potential and water pH. Increases in phosphorus concentrations were likely due to physical changes to the sediment environment, and increased transport of dissolved phosphorus from sediment porewater to surface waters. Management of ditches to reduce delivery of phosphorus to

receiving waters should consider effects of burrowing benthic invertebrates on physical and biogeochemical processes at the sediment-water interface.

Introduction

Healthy, functioning stream ecosystems have intact structural components such as habitat and biodiversity providing functions within the environment to sustain the ecosystem against environmental perturbations (Palmer and Febria 2012). Understanding the relationship between biodiversity and the ecosystem functions they produce is important for maintaining healthy ecosystems and prioritizing conservation strategies to preserve specific functions (Hooper et al. 2005). Studies from terrestrial systems show that increasing biodiversity generally has positive effects on ecosystem services (Balavanera et al. 2006). In aquatic ecosystems, that relationship is not always straightforward, as there can be significant effects of the environment mediating the effect of different species on ecosystem functions (Vaughn 2010, Covich et al. 2004, Cardinale and Palmer 2002, Lecerf and Richardson 2010). Some species may produce disproportionate effects on functions in an ecosystem, such as ecosystem engineers and keystone predators (Moore 2006, Woodward et al. 2008, Keitzer and Goforth 2013). Some combinations of species may produce non-additive effects on ecosystem functions, such as facilitation between two species (Cardinale et al. 2002). Understanding species-specific effects on ecosystem functions across a range of environmental conditions is needed before accurate predictions can be made about the relationship between biodiversity and ecosystem functions.

In stream ecosystems, macroinvertebrate species play important roles in the transformation and transport of materials and energy from headwaters to downstream

bodies of water (Wallace and Webster 1996). For many species of aquatic macroinvertebrates, their functions in ecosystem-level processes are best studied and quantified in relation to their feeding behaviors and trophic position (Cummins and Klug 1979, Merritt et al. 2008). Grouping species into these functional feeding groups allows predictions to me made about the effects different species have on processes such as decomposition, primary production, and nutrient cycling (Lavorel and Garnier 2001). The healthy functioning of pristine stream ecosystems can be inferred in part by the presence of these functional feeding groups (Cummins 1974, Karr and Chu 1998, Davies and Jackson 2006).

Bioturbation is the disruption of aquatic sediments by the burrowing behaviors of aquatic invertebrates, and is another function provided by aquatic macroinvertebrates that has been less well studied (Freckman et al. 1997, Palmer 1997, Covich et al. 1999). The effects of bioturbation in aquatic and marine environments and their importance to the healthy functioning of streams and other bodies of water are an active area of research (Meysman et al. 2006). Bioturbation by burrowing benthic invertebrates has been found to alter rates of nutrient cycling (Michaud et al. 2006, Chaffin and Kane 2010), recruitment of species in dormant stages (Ståhl-Delbanco and Hansson 2002), and secondary productivity by sediment microbes (Nogaro et al. 2008). Attempts to classify burrowing macroinvertebrates into functional groups have focused on the nature of the physical change that species produce to the sediment habitat, rather than on the effects those physical changes produce on ecosystem functions (Gerino et al. 2003). Studies have shown that the effects of bioturbation on ecosystem processes can be complex, with strong effects of environmental context, and variability between species of the same

functional group (Roskosch et al. 2012, Mermillod-Blondin and Rosenberg 2006, Mermillod-Blondin et al. 2001, Michaud et al. 2006).

Drainage ditches are a type of aquatic habitat where bioturbation may play an important role in regulating nutrient exchanges between the sediment and surface water. As a part of agricultural infrastructure, ditches are not maintained or regulated as natural bodies of water, and their primary function is the conveyance of water from lands that would otherwise be unable to support crops (Grumbles 1991). Nevertheless, ditches do provide aquatic habitat to many different species of plants and animals, and some research has found that ditches may also function as important sources of biodiversity, as the only non-cropped areas within intensely agricultural landscapes (Herzon and Helenius 2008, Verdonschot et al. 2011). Current research has also focused on the potential of ditches to function as mitigation wetlands, where biogeochemical processes occurring primarily within ditch sediment improve the quality of water draining from agricultural fields (Hill and Robinson 2012, Kröger et al. 2013, Usborne et al. 2013). So far, few studies to date have recognized the potential for the macroscopic biological communities within ditches to have an effect on beneficial biogeochemical processes occurring within sediments (but see Vaughan et al. 2008, Needelman et al. 2007b).

The current study investigates the effects that burrowing species have on phosphorus exchange between sediment and surface water in drainage ditches. The transport of phosphorus by drainage ditches is of concern to resource managers in Maryland due to its role in eutrophication of the Chesapeake Bay and the decline of benthic shellfish industries that have resulted from hypoxic “dead zones” that form after seasonal algal blooms (Boesch et al. 2001, Hagy et al. 2004, Russell et al. 2008). Current

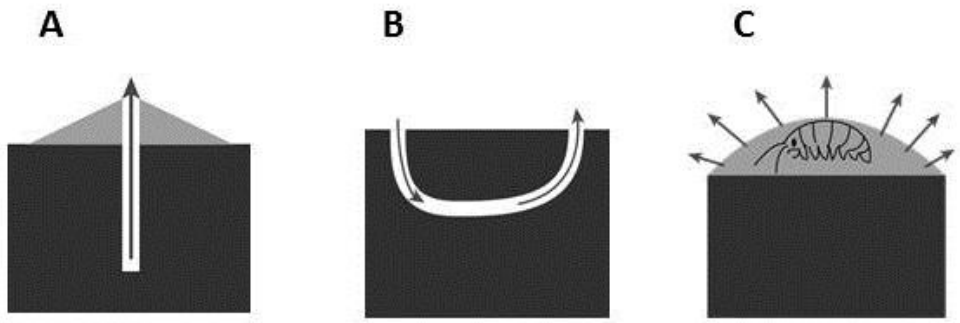
farm management techniques limit the amounts of phosphorus applied to fields to meet nutrition requirements of crops without causing excess to runoff, but a long history of manure application in the area has produced high background concentrations of phosphorus in the soil, which increases its mobility in water draining from this land (Kleinman et al. 2007). Gradients of oxidation-reduction potential that develop across the sediment-water interface of drainage ditches provide a highly reactive site for chemical transformations in water that discharges into drainage ditches (Needelman et al. 2007b). Previous studies have shown that bioturbation by benthic invertebrates can increase (Mermillod-Blondin et al. 2005, Chaffin and Kane 2010), decrease (Lewandowski 2007), or have no effect (Mermillod-Blondin et al. 2005) on phosphorus movement from sediment to water, depending on the type of burrowing and environmental contexts.

There are many biogeochemical processes occurring across the sediment-water interface of ditches that determine whether phosphorus is retained within the sediment or sediment pore water or released to the surface water. Phosphorus can be adsorbed to surfaces such as iron oxides, clay films, and organic matter where it can be easily exchanged with phosphorus in solution (Mackie 2004). Sharp redox gradients often exist at the surface of drainage ditch sediments, and anaerobic conditions may exist close to the surface where iron oxide coatings can be reduced, releasing adsorbed phosphorus (Leslie et al. 2012, Venterink et al. 2002). Reduced iron may become oxidized at the surface, forming poorly crystalline ferrihydrite, which may adsorb some of the released phosphate (Axt and Walbridge 1999). Phosphorus can form complexes with iron, aluminum, or calcium and precipitate as secondary minerals (Dunne and Reddy 2005). Drainage ditches on Maryland's Eastern Shore often have sulfidic materials present at or

below the surface, which may compete with dissolved phosphorus to bond with iron, forming iron monosulfides (Maynard et al. 2011, Vaughan et al. 2008). Microbial biofilms and plant roots can take up some forms of reactive phosphorus directly from solution where it can be stored as microbial and plant biomass (Kerrn-Jespersen and Henze 1993, Huang et al. 2011, Huett et al. 2005).

Burrowing by different species of macroinvertebrates creates different types of disturbance within sediments, which may have different effects on specific sediment-phosphorus processes. Burrows may create preferential flow paths that keep pore water from interacting with some reactive surfaces within the sediment (Nogaro et al. 2006). Irrigation of burrows may alter redox potentials near the surface by bringing water carrying dissolved oxygen into subsurface sediment layers (Hunting et al. 2012). Deposit-feeding organisms may redistribute clay-sized particles through selective feeding, and may directly alter microbial biomass through grazing (McCall and Fisher 1980). Differences in the physical effects that different species have on the sediment have been used to classify burrowers into different functional groups based on the nature of the mechanical disruption they create (Gerino et al. 2003). Conveyors (Fig. 4.1a) are deposit-feeding organisms that ingest sediment particles at depth, and deposit egested sediment at the surface, creating a net-upward movement of sediment. Gallery-diffusors (Fig. 4.1b) occupy burrows that are kept aerated by moving currents of water through the tunnels. Biodiffusors (Fig. 4.1c) are organisms that do not necessarily construct defined burrows, but live or forage within surface sediments, causing surface sediments to be randomly scattered and mixed.

Figure 4.1 Diagrammatic representation of disturbance caused by three functional groups of bioturbation. (A) Conveyors feed at depth and deposit egested sediment at the surface. (B) Gallery-diffusors irrigate burrows with surface water. (C) Biodiffusors randomly scatter surface sediments.



The objective of this study was to determine the effects that different types of bioturbation have on phosphorus exchange between ditch sediment and water across a range of environmental conditions. Microcosms were used to test the effect of different functional groups of burrowers on phosphorus exchange between sediment and surface water, using different species of macroinvertebrates to produce different types of bioturbation. Species were chosen based on their relative abundance in previous studies to represent the dominant burrowing species in ditches, and to represent different functional groups of bioturbation (*sensu* Gerino et al. 2003). To simulate different environmental contexts within drainage ditches, sediment and water were manipulated during the experiments. One of the primary factors determining macroinvertebrate community composition in drainage ditches is whether the ditch is a large perennial or a small temporary body of water. One of the differences between small and large ditches is the development of granular structure in the sediment in small ditches that results from cycles of wetting and drying, and the single-grained sediment in large ditches, which tend to hold water year-round (Vaughan et al. 2008). The interaction of different species with

different sediment structures was investigated to determine whether effects of bioturbation on phosphorus dynamics are mediated by the physical structure of the sediment habitat. Water within ditches may alternate between periods of stagnation and flow depending on the position of the water table. Therefore, measurements of phosphorus were made during different periods of water movement and stasis within microcosms to determine the role of hydrology in mediating the effect of bioturbation on phosphorus dynamics. My hypothesis is that species of burrowing invertebrates will alter the concentration of phosphorus in the surface water relative to controls by altering the porosity and redox chemistry of sub-surface sediments through burrow construction and ventilation, and through direct effects on the microbial biomass through grazing.

Methods

Sediment collection and preparation

Sediment was collected from a single perennial ditch located in Caroline County, on Maryland's Eastern Shore. This ditch is a tributary of Mason Branch, within the Choptank River watershed. Sediments were collected where the ditch is draining soils in the Hurlock (coarse-loamy, siliceous, semiactive, mesic Typic Endoaquults) and Falsington (fine-loamy, mixed, active, mesic Typic Endoaquults) series map units. For each experiment, approximately 20 L of surface sediments (approximately 15 cm deep) were randomly collected between 14 Oct 2013 and 4 Feb 2014 prior to each microcosm experiment. Sediments were homogenized, removing large pieces of organic matter and dried at 60°C for 72 hours. Drying was an effective way of defaunating sediments prior to their use in experiments, and provided structure that could then be manipulated for different treatments. Dry sediments were manually crushed and sieved to two different sizes. Sediments were first passed through a 4 mm sieve, which gave a product with

coarse aggregates of soil material approximating the structure found in small ditches that are dry seasonally, and have actively developing soil structure. A subset of those coarse sediments were then crushed with a mortar and pestle, and passed through a 500 µm sieve. This fine sediment approximates the loose, unconsolidated sediments found in larger perennial ditches that do not often dry or develop soil structure.

For each experiment, a subset of the sediment used for both the fine and coarse sediment treatments was analyzed for pH, water-extractable phosphorus, and organic matter content. Three replicates were measured for pH using a 1:1 slurry of 10 g of sediment and 10 mL of deionized water. An additional 90 mL of deionized water was added to this slurry and shaken to extract water-soluble phosphorus. The slurry was then vacuum-filtered through Whatmann #1 filter paper, and analyzed along with water samples collected from microcosms for soluble, reactive phosphorus. Organic matter content of sediment was measured from five replicates of each type of sediment as the difference in mass of dry sediment samples measured before and after burning off organic matter in an oven at 500°C for 24 hours.

Microcosm construction

Experimental microcosms were constructed to allow aquatic macroinvertebrates to burrow within a layer of anaerobic, aquatic sediments with a layer of oxygenated water above, and water moving through the sediment layer to be discharged at the surface, resembling conditions in Maryland drainage ditches. To construct the microcosms, 600 mL glass beakers were filled with 150 g of acid-washed pea gravel, and 100 g of either fine or coarse prepared sediments. For the first experiment, microcosms were filled with dechlorinated tap water and subsequent experiments used standard, synthetic, moderately

hard freshwater (US EPA 2002a). Microcosms had a hydraulic connection to a 200 mL reservoir beaker through a piece of aquarium tubing embedded within the gravel layer of the microcosm. To keep the aquarium tubing from shifting within the sediments during the experiment, it was housed within a section of 9.5 mm diameter PVC tubing, attached to the side of the 600 mL beaker with a metal clip. By raising the reservoir so that its water level was above that of the microcosm, the difference in hydraulic head allowed water to move through the aquarium tubing and through the sediments, to be discharged at the surface of the microcosm.

Each microcosm had three platinum-tipped electrodes embedded just below the sediment surface to measure oxidation-reduction (redox) potential. Electrodes were constructed using a short piece (approximate 15 mm) of 0.5 mm diameter Pt wire inserted into one end of a piece of copper capillary tubing (1.24 mm I.D. × 2.51 mm O.D. × 300 mm) where it was crimped into place, exposing approximately 10 mm of Pt wire. The entire length of copper tubing was covered in 3.175 mm diameter heat-shrink tubing, exposing 2 cm of copper tubing on the end opposite the Pt wire. The Pt-Cu junction was then sealed using waterproof epoxy, so that only the Pt wire was exposed to the sediment environment when inserted into the microcosm. Each electrode was tested using an oxidation-reduction probe standard solution (Light's solution) before and after each experiment (Light 1972).

For each experiment, twenty microcosms were constructed, and kept in a walk-in environmental chamber with the temperature set at 20°C, and a 14:10 day/night cycle. Surface water was kept oxygenated by bubbling air through a Pasteur pipette inserted just below the surface of the water. Each pipette was connected via a section of aquarium

tubing to a central manifold, pressurized by an electric air pump. Valves located at each connection point controlled air flow to ensure that turbulence from bubbling did not disturb sediments within the microcosms. Water levels in the microcosms were examined daily, and were topped off with deionized water to replace water lost to evaporation. Microcosms were wrapped in aluminum foil to block light from reaching the sediments and stimulating photosynthesis below the sediment surface. After the first experiment, each microcosm was enclosed in a clear plastic cylinder with the top covered in insect netting, and a cut-out window to allow access to the microcosm. This covering prevented any unwanted insects from colonizing the microcosms over the course of the experiment.

Invertebrate collection

Four different species of burrowing invertebrates were used in four separate experiments, representing three different bioturbation functional groups: conveyors, biodiffusors, and gallery-diffusors. Invertebrates were collected at different dates from the same ditch where sediments were collected, as well as some nearby ditches. On each sampling date, organisms were collected using a D-frame net (500 µm mesh, 0.3 m^2 opening) to sweep through the ditches until two 19 L buckets were filled with material All material was returned to the laboratory, where it was stored at 20°C and kept aerated to allow organisms to acclimate to temperature conditions of the microcosms. Just prior to adding organisms to microcosms, samples were rinsed in a 500 µm sieve, and organisms were collected without magnification. For each experiment, the densities of organisms simulated the densities of individuals measured from quantitative surveys of burrowing invertebrates (Ch. 3). However, the densities used in experiments were constrained in some cases by the number of individuals available from sampling.

The first two experiments used two different species of aquatic worms (Haplotaxida: Naididae). The first species, *Ilyodrilus templetoni* (Southern), is an oligochaete that is predominantly associated with larger drainage ditches, and can be distinguished on the basis of its small size and long hair chaetae. The second species, *Limnodrilus hoffmeisteri* (Claparede), is an oligochaete that is cosmopolitan in distribution and ubiquitous across types of drainage ditches in Maryland, and it can be recognized by its larger size and lack of long, hair chaetae. Both species feed on silt and clay-size particles at depth, and egest feces at the sediment's surface, and therefore belong to the conveyor functional bioturbation group. *I. templetoni* worms were introduced to microcosms at a density of 100 individuals per microcosm, while *L. hoffmeisteri* were introduced to microcosms at a density of 50 individuals per microcosm. The third experiment used the freshwater crustacean *Crangonyx* sp. (Amphipoda: Crangonyctidae). This genus of amphipod is primarily associated with small drainage ditches, but is not exclusive to small ditches. Amphipods such as *Crangonyx* do not build defined burrows, and instead randomly scatter surface sediments as a result of feeding on detritus within the surface of the sediment-water interface, and therefore belong to the biodiffusor bioturbation functional group. The fourth experiment used the aquatic larvae of the midge *Chironomus decorus* S.G. (Diptera: Chironomidae). The larvae of this genus of fly are cosmopolitan in distribution, and are found in nearly all aquatic habitats. In drainage ditches, larvae of this species are found primarily in larger collection ditches, and can be identified by their bright, red color and the presence of two pairs of ventral tubules on the terminal abdominal segment. Larvae of midges like *C. decorus* build U-shaped burrows within aquatic sediments, and keep those burrows aerated as they feed on

small detritus and suspended microbes. Therefore, they are classified within the bioturbation functional group of gallery-diffusor.

Sampling structure

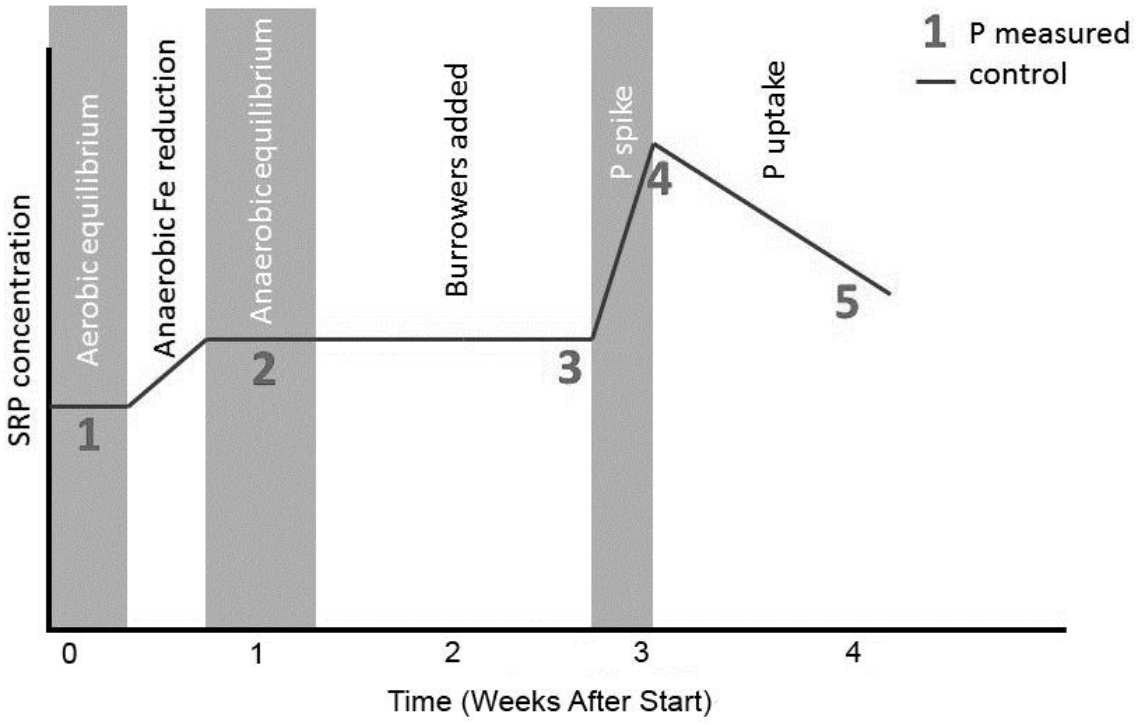
Samples of surface water (~100 mL) were taken from microcosms using a 60 mL syringe fitted with a short length (~15 cm) of aquarium tubing. Water was carefully siphoned from just below the surface of the water level within the microcosm to avoid disturbing the sediments below. The volume of water removed was replaced with dechlorinated tap water or synthetic freshwater directly to the microcosm, and not through the reservoir. Water samples were vacuum-filtered through Whatmann #1 filter paper, and stored in 125 mL polyethylene bottles at -20°C prior to analysis. Concentration of soluble, reactive phosphorus (SRP) was determined from a 50 mL aliquot of the total sample using the ascorbic acid method (Greenberg 1995). The ascorbic acid method is a colorimetric analysis, where the absorbance at 880 nm of the end product is measured using a spectrophotometer (Spectronic 20D). For each set of samples, ten standard reference concentrations (0 – 2.5 mg/L PO_4^{3-}) were also analyzed to develop a standard curve. A linear least-squares regression was fit through the plot of references, and the slope and intercept of the line were used to convert the absorbance measurements of the samples to concentrations.

Water samples were taken at five points during the course of each experiment, to measure five distinct stages of phosphorus movement between the sediment and water phases (Fig. 4.2). The first measurement was taken within a day of the construction of the microcosms (0-1 days after start), as a measure of the concentration of soluble phosphorus extracted by the water moving through the sediment layer. The second water

sample was taken about a week after the construction of the microcosms (6-10 days after start). This was a measure of the change in concentration of phosphorus in the microcosms related to the release of phosphates as a result of anaerobic reduction of iron oxides, and was also the time at which burrowing invertebrates were added to half of the microcosms. The third water sample was taken about two weeks after the introduction of the burrowing invertebrates (19-22 days after start, 10-15 days after introduction of burrowers). This was a measure of the new phosphorus concentration that resulted from bioturbation. The fourth water sample was taken shortly after the third (21-27 days after start, 2-5 days after third measurement), and was preceded by a complete water change in the microcosms with water containing 1.0 mg/L PO_4^{3-} as a way of simulating the subsurface flow and discharge of high-nutrient water that occurs in drainage ditches. To accomplish this, the aquarium tubing used to fill the microcosm from the reservoir was pinched shut, and the reservoir was replaced with a new 250 mL beaker containing water spiked with phosphate. Before opening the tubing, all of the surface water in the microcosm was decanted off using a syringe. After opening the tubing, water flowed from the reservoir and into the microcosm, displacing sediment porewater that discharged to the surface. After approximately 300 mL of water discharged to the surface, the water was also decanted off using the syringe, to avoid mixing of the new phosphate-spiked water with sediment porewater. Microcosms were then slowly filled with phosphate-spiked water through the reservoir before taking the fourth water sample. This was a measure of the capacity of the sediment to rapidly take-up dissolved phosphate, possibly though adsorption to surfaces, and the differential adsorption rates between treatments. The fifth and final water sample was taken about a week after the water replacement and

fourth water sample (29-36 days after start, 8-10 days after water replacement and fourth measure). This was a measure of the differential uptake of phosphate from the water as a result of processes that are slower than rapid adsorption, such as uptake and incorporation into microbial biomass.

Figure 4.2 Hypothesized relationship between SRP concentration and time for experimental microcosms, and numbers indicating points where water samples were collected. The increase in SRP from 1 to 2 occurs from release of P sorbed to iron oxides that are reduced by anaerobic bacteria. Changes between 2 and 3 may occur from bioturbation by burrowing invertebrates. SRP may change from 3 to 4 as SRP is added to the system. SRP levels are expected to drop between 4 and 5 as added P is taken up through different biogeochemical processes.



Redox potential and pH were measured using electrodes inserted into the microcosms. Redox was determined using a hand-held multimeter (RadioShack model 22-810), modified according to Rabenhorst (2009) with a TL082 dual JFET operational amplifier (Texas Instrument model TL082ACP) and a calomel reference electrode (Accumet model 13-620-259) that was inserted into the surface water of the microcosm.

A temperature-specific correction factor of +243 mV was added to each measured potential to convert measures to be relative to a standard hydrogen electrode.

Statistical analyses

The four experiments were each completely randomized designs with a crossed factorial treatment structure. Each experiment had two factors under investigation (sediment size and burrowing invertebrates), and each factor had two levels (coarse/fine, present/absent, respectively), for a total of four treatment combinations per experiment. Phosphate concentrations and redox potentials were repeatedly measured from each microcosm, therefore any statistical comparisons made across sampling dates using time as a third factor had to take into account the non-independence of observations. Since there were some differences in the sediment source and timing of different steps in the course of the experiments, direct comparisons between effects of different organisms could not be made. More meaningful comparisons could be made between the control treatments of different experiments, and between the first and second water samples, before any burrower treatments were applied. In each case, ANOVA was used to compare means after all assumptions of the test were checked, mainly normal distribution of residuals and homogeneity of variances.

Mean values for pH, water extractable phosphorus, and organic matter content of the sediment used for the four different experiments were compared using ANOVA to determine whether there were any differences in these parameters for any of the experiments, and to determine whether there were any consistent differences in these parameters between the two sediment sizes. To determine the effects of sediment size and anaerobic reduction of iron on phosphorus release, the mean phosphorus concentrations

were compared between the first and second water samples using ANOVA with sediment size and time as factors. Since the first and second measurements were taken from the same microcosms, the data were analyzed as a split-plot design.

To determine the effect of bioturbation by burrowing invertebrates and sediment grain size on phosphorus dynamics, comparisons were made between the four treatment combinations on each sampling date, after the addition of burrowing invertebrates. To do this, separate ANOVAs were done for each sampling date, using sediment size, burrower presence, and their interaction as explanatory variables. Where there was a significant interaction, planned orthogonal contrasts were used to test for differences between treatments. Comparisons between treatment combinations that differed in both factors were omitted from contrasts.

To determine whether bioturbation by burrowing invertebrates and sediment size had an effect on pH and redox chemistry of the sediment, comparisons were made between the treatments on each sampling date. Sediment size was the only factor used in the models before burrowers were added, and then sediment size, burrower presence, and their interaction were included as terms in the model after the addition of burrowers. For the analysis of redox, the mean of the three measurements of redox potential per microcosm was used as the response variable. To determine whether there was an effect of either of these factors on heterogeneity of redox potentials in the sediment, the coefficient of variation was calculated for the three redox measures at each sampling date, and the same statistical models were repeated, using the coefficient of variation as the response variable. Where significant differences were found in phosphorus concentrations between treatments, regression analysis was used to determine whether

pH, the measured redox potential, or variance in redox potential could explain a significant proportion of the variance in phosphate concentration.

All analyses were performed using R v 3.1.0 (R Core Team 2014). Linear mixed-effects models were built using the package *nlme* (Pinheiro et al. 2014). ANOVAs were performed using the *car* package (Fox and Weisberg 2011). Matrices for orthogonal contrasts were coded using the package *contrast* (Kuhn et al. 2013). Pairwise means comparisons were performed using the package *multcomp* (Hothorn et al. 2008). Graphics were produced using the package *ggplot2* (Wickham 2009).

Results

Sediment qualities

Sediment pH was significantly affected by the source of sediment and the size class to which it was crushed, with a significant interaction between the two factors (size: F=11.34; df=1, 16; P=0.004; source: F=56.48; df=3, 16; P<<0.001; interaction: F=13.81; df=3, 16; P<0.001). Pairwise comparisons of individual treatment combinations show that the largest difference in mean pH between any two samples of sediment is 0.2, and that there are no consistent differences between size classes or sources of sediment (Table 4-1). There are significant differences in the sediment organic matter content (measured as mass lost on ignition) between sources of sediment and size classes, with no significant interaction term (size: F=32.564; df=1, 32; P<<0.001; source: F=53.296; df=3, 32; P<<0.001; interaction: F=0.847; df=3, 32; P=0.479). Sediment used for the *C. decorus* experiment had significantly less organic matter present compared to the other three experiments (mean of 2.96% less than other experiments, Table 4-1). The coarse sediment had on average 1.16% less organic matter present than the fine sediment. There

were differences in water-extractable phosphorus between different sources of sediment, but there were no consistent differences between size classes across the different experiments (size: F=0.043; df=1, 15; *P*=0.838; source: F=16.582; df=3, 15; *P*<<0.001; interaction: F=3.010; df=3, 15; *P*=0.063).

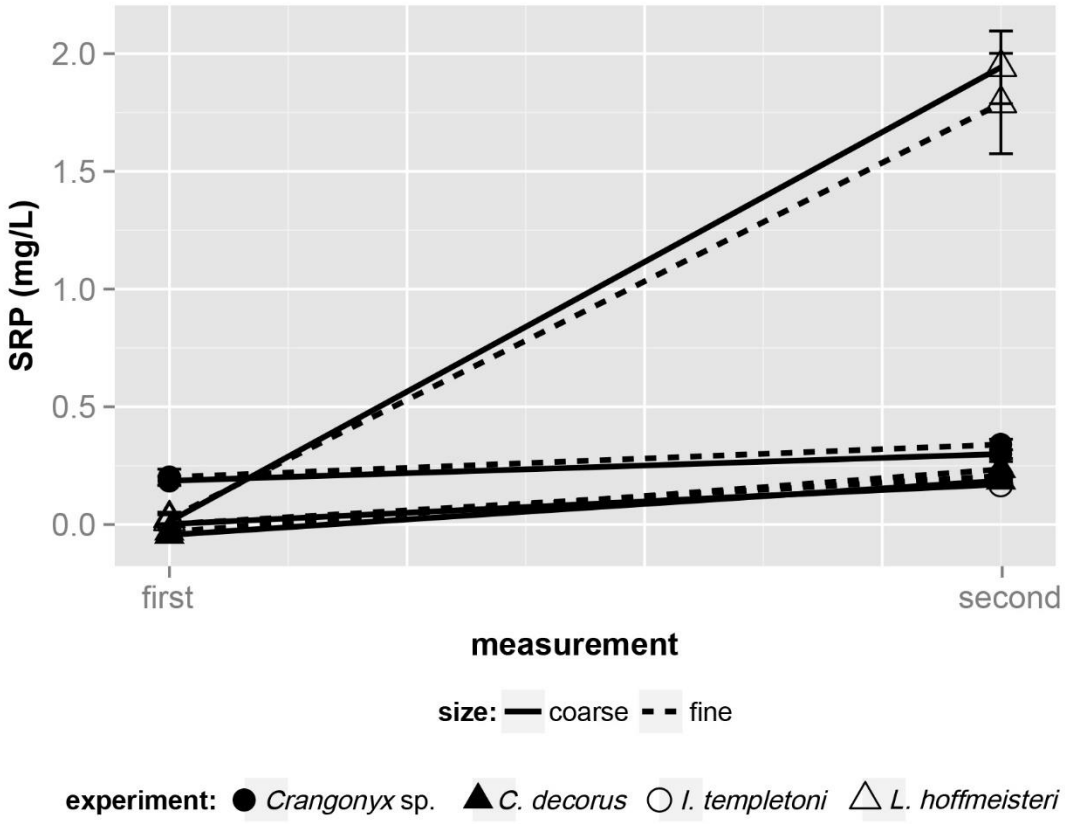
Table 4-1 Initial measurements of sediment pH, % organic matter content (%O.M.) and water extractable SRP from sediment used for each experiment.

Experiment	**Size**	**pH**	**%O.M.**	**SRP (mg/L)**
I. templetoni	coarse	4.7	11.1	0.48
I. templetoni	fine	4.7	12.6	0.72
L. hoffmeisteri	coarse	4.7	10.7	0.17
L. hoffmeisteri	fine	4.6	12.1	0.07
Crangonyx sp.	coarse	4.6	11.1	0.40
Crangonyx sp.	fine	4.6	11.8	0.29
C. decorus	coarse	4.5	8.2	0.24
C. decorus	fine	4.6	9.1	0.40

Initial P measurements

For each experiment, there were greater concentrations of SRP measured on the second sampling point than the first sampling point (Fig. 4.3). The mean SRP at the onset of experiments was 0.044 mg/L and 0.646 mg/L after the second measurement. For the *I. templetoni* experiment, there was also a significant effect of sediment size and a significant interaction between sediment size and measurement on the concentration of SRP (sediment: χ^2=3.6; df=1; *P*=0.0578; interaction: χ^2=3.6; df=1; *P*=0.0578). For this experiment, the concentration of SRP was higher in microcosms with fine sediments, but only at the second sampling point.

Figure 4.3 First two measures of SRP concentration in microcosms before the addition of burrowers. Shapes represent measurements from different experiments: closed circle – Crangonyx sp.*, closed triangle –* C. decorus*, open circle –* I. templetoni*, open triangle –* L. hoffmeisteri*. Solid lines represent coarse sediment and dashed lines represent fine sediment treatment.*



Differences in P from bioturbation

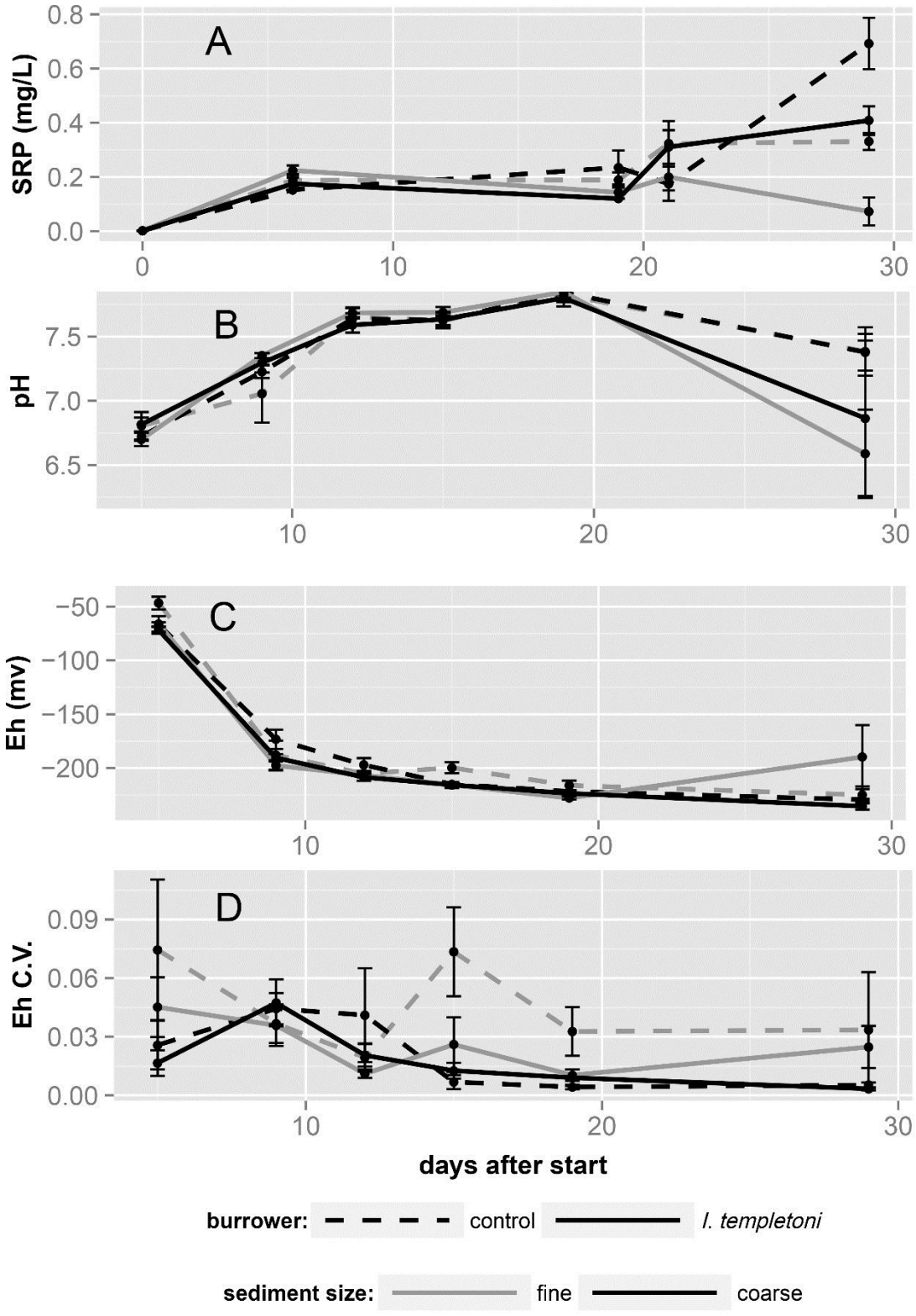
1. I. templetoni *(conveyor)*

At 19 days after start, and 13 days of burrowing by *I. templetoni*, there was no effect of sediment size on the mean SRP concentration, but the effect of burrowers was marginally significant at an alpha of 0.05 (i.e. P=0.06) (Fig. 4.4a). Comparisons of the means of individual sediment treatments between burrower effects show both burrower treatments had slightly less SRP than controls, with a difference of 0.078 mg/L. After flushing microcosms with water containing 1.0 mg/L PO_4 at 21 days after start, there

were no significant differences in SRP concentration between treatments. After eight days of incubation, SRP concentration was higher in microcosms with coarse sediment than fine sediment ($F=30.153$; $df=1, 15$; $P<<0.001$) and SRP concentrations were lower in microcosms receiving *I. templetoni* than controls ($F=18.060$; $df=1, 15$; $P<0.001$) with no significant interaction between factors. There was not a significant relationship between SRP concentrations and pH, mean redox potential, or variation in redox potential within a microcosm for sample dates where burrowers had a significant effect on phosphorus concentrations.

Burrower and sediment treatments had significant effects on pH, redox potential, and variation in redox potential on different sampling dates (Fig 4.4b,c,d). At five days after start, microcosms with coarse sediment had lower mean redox potentials than microcosms with fine sediment ($F= 4.104$; $df=1, 17$; $P=0.059$). At 15 days after start, there were significant effects of sediment ($F=5.792$; $df=1, 15$; $P=0.029$), burrowers ($F=6.931$; $df=1, 15$; $P=0.019$), and their interaction ($F=5.947$; $df=1, 15$; $P=0.028$). Pairwise comparison of treatment means shows that microcosms with *I. templetoni* have lower redox potentials ($t=3.588$; $P=0.005$), but only for the fine sediment treatment, and coarse sediment has lower redox potentials than fine sediment ($t= 3.424$; $P=0.007$), but only among no-burrower controls. There is also a significant effect of sediment size on the variation in redox measurements within a microcosm at day 15, with fine sediments being more variable than coarse sediments ($F= 7.916$; $df=1, 15$; $P=0.013$). At 19 days after start there is a significant effect of *I. templetoni* on redox potential ($F= 8.058$; $df=1, 15$; $P=0.012$), and the interaction between *I. templetoni* and sediment size is marginally significant ($F= 3.844$; $df=1, 15$; $P=0.069$). Pairwise means comparisons of the effect of

Figure 4.4 Response variables measured from microcosms with I. templetoni *(conveyor): (A) soluble reactive phosphorus concentrations, (B) pH, (C) redox potential, (D) coefficient of variation for replicate redox measures within a microcosm.*



burrowers across the two levels of sediment shows that microcosms with *I. templetoni* have lower redox potentials only in the fine sediment treatments (t= 3.411; *P*=0.008). Sediment size (F= 4.327; df=1, 15; *P*=0.055) and the interaction of sediment size and burrowers (F= 4.173; df=1, 15; *P*=0.059) had a significant effect on the variation in redox measurements within a microcosm at 19 days after start. Pairwise means comparisons of the effect of sediment size at both levels of burrower treatment show that microcosms with fine sediment had more variation in redox measurements, but only among no-burrower controls (t=2.915; *P*=0.021). There was a significant effect of *I. templetoni* on pH at 29 days after start, with microcosms containing burrowers having a lower pH than controls (F=4.3614; df=1,15; *P*=0.054) (Fig. 4.4b).

2. L. hoffmeisteri *(conveyor)*

At 20 days after start and 10 days of burrowing by *L. hoffmeisteri*, there was no effect of the burrower treatment on SRP concentration of surface water, while microcosms with coarse sediment continued to have more SRP than microcosms with fine sediment (F=6.031; df=1, 16; *P*=0.026) (Fig.4.5a). After flushing microcosms with water containing 1.0 mg/L PO_4 at 22 days after start, microcosms with coarse sediment continued to have significantly higher SRP concentrations than microcosms with fine sediment (F=33.379; df=1, 16; *P*<<0.001), and microcosms with *L. hoffmeisteri* had significantly higher SRP concentrations than microcosms without burrowers (F=4.414; df=1, 16; *P*=0.052). Redox potential and pH did not have a significant relationship with SRP concentration. After seven days of incubation, microcosms with coarse sediment continued to have higher SRP concentrations than microcosms with fine sediments (F=33.8; df=1, 16; *P*<<0.001), but the effect of burrowers was reversed, as microcosms with *L. hoffmeisteri* had lower SRP concentrations than control microcosms (F=22.4;

df=1, 16; *P*<0.001). Again, there was not a significant relationship between redox potential and SRP concentration. There was a significant linear relationship between pH and SRP concentration at 29 days after start, with higher SRP concentrations measured in microcosms with higher pH values (R^2=0.35; *P*=0.006) (Fig 4.6). Redox potential was higher in microcosms with *L. hoffmeisteri* (F= 4.511; df=1, 16; *P*=0.050) only on the last day of redox measurement (27 days after start) (Fig. 4.5c). There was no difference in variation in redox measurements within a microcosm between any of the treatment combinations on any of the sampling dates (Fig. 4.5d). At 27 days after start there is a significant effect of *L. hoffmeisteri* on pH, with microcosms receiving burrowers having lower pH than controls (F=27.00; df=1, 16; *P*<<0.001) (Fig. 4.5b).

3. Crangonyx *sp. (biodiffusor)*

At 22 days after start and 15 days of burrowing by *Crangonyx*, there was no effect of burrower treatment on SRP concentrations, but microcosms with coarse sediment had higher SRP concentrations than microcosms with fine sediment (F=6.065; df=1, 16; *P*=0.026) (Fig. 4.7a). After flushing microcosms with water containing 1.0 mg/L PO_4 at 27 days after start, microcosms with coarse sediment had higher SRP concentrations than microcosms with fine sediment (F=16.411; df=1, 16; *P*<0.001), and microcosms with *Crangonyx* had lower SRP than control microcosms (F=4.149; df=1, 16; *P*=0.059). SRP concentrations had a significant relationship with measured redox potentials (R^2=0.27, *P*=0.018), with lower SRP concentrations in microcosms with higher redox potentials (Fig. 4.8). There was no significant linear relationship between pH and SRP concentration at 27 days after start. After nine days of incubation, microcosms with

Figure 4.5 Response variables measured from microcosms with L. hoffmeisteri *(conveyor): (A) soluble reactive phosphorus concentrations, (B) pH, (C) redox potential, (D) coefficient of variation for replicate redox measures within a microcosm.*

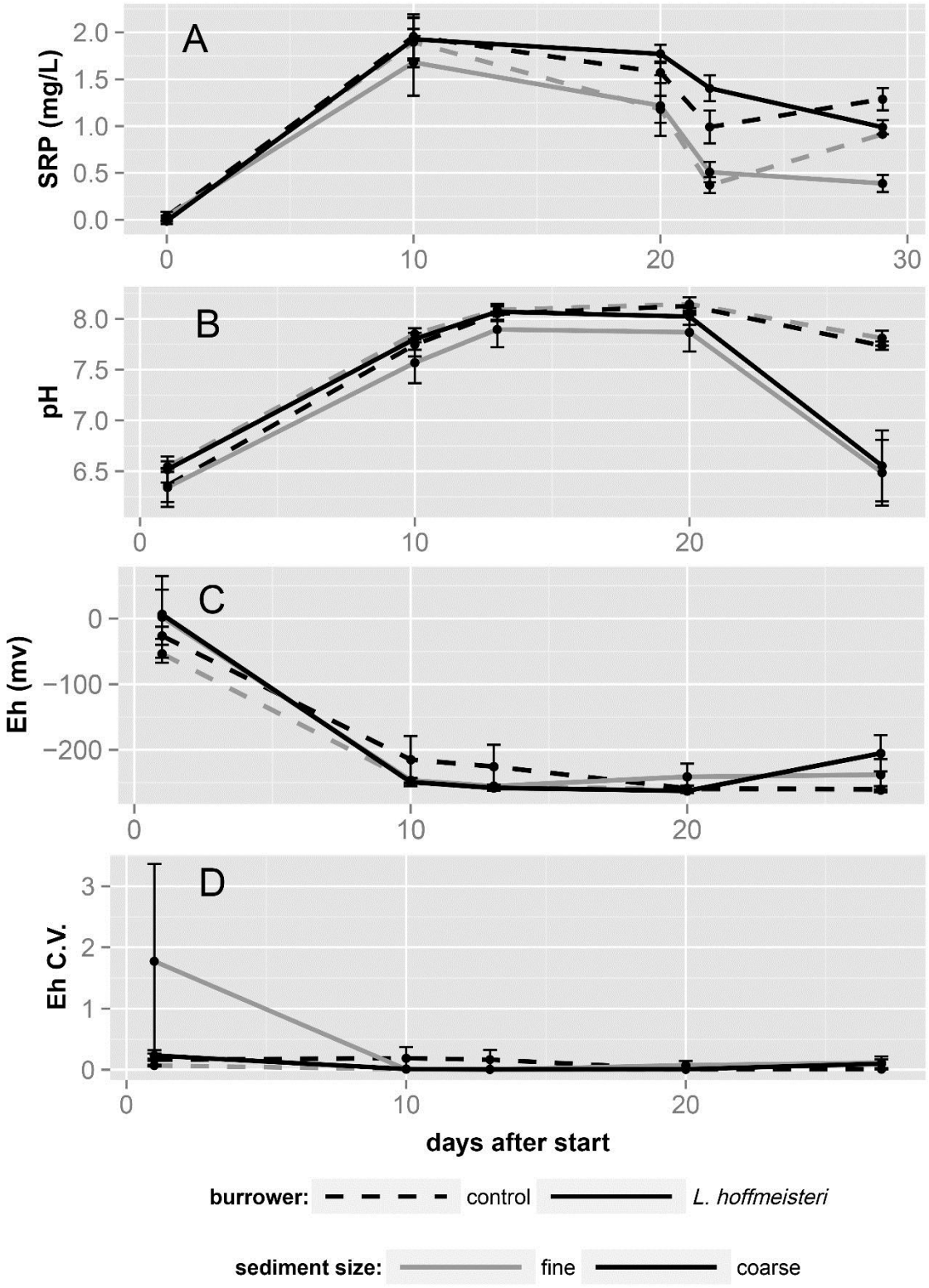
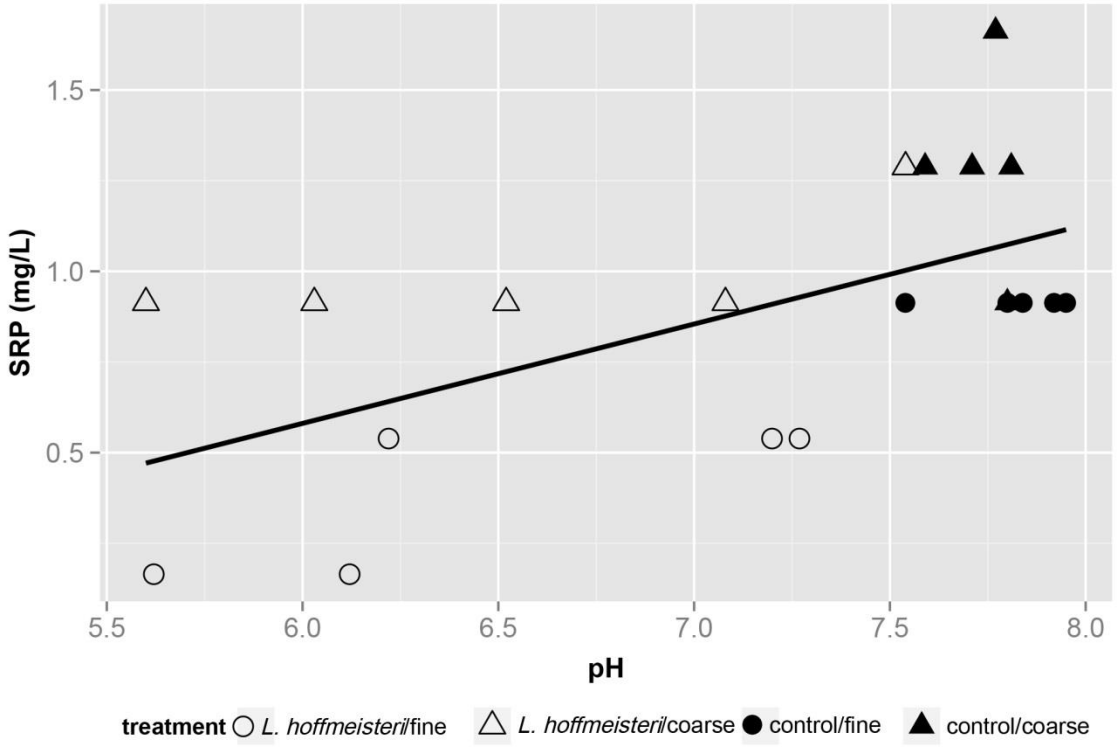


Figure 4.6 Linear relationship between SRP measured after a week of incubation following flush with 1mg/L PO_4 (29 days after start). Line represents least-squares regression (R^2=0.35, P=0.006).



coarse sediment had higher SRP concentrations than microcosms with fine sediment (F=5.753; df=1, 16; *P*=0.029), and there was no effect of burrowers on SRP concentrations. *Crangonyx* had a significant effect on redox potential in the sediment at 22 (F=7.275; df=1, 16; *P=0*.016) and 27 (F=5.3134; df=1, 16; *P*=0.035) days after start (Fig. 4.7c). At both sampling dates, microcosms with *Crangonyx* had higher measured redox potentials than controls. *Crangonyx* also had a significant effect on pH at 22 (F=25.779; df=1, 16; *P*<0.001) and 27 (F=22.264; df=1, 16; *P*<0.001) days after start. At both sampling dates, pH was lower in microcosms with *Crangonyx* than controls (Fig. 4.7b). There were not differences in the coefficient of variation between any treatment combinations at any date (Fig. 4.7d).

Figure 4.7 Response variables measured from microcosms with Crangonyx sp. *(biodiffusor): (A) soluble reactive phosphorus concentrations, (B) pH, (C) redox potential, (D) coefficient of variation for replicate redox measures within a microcosm.*

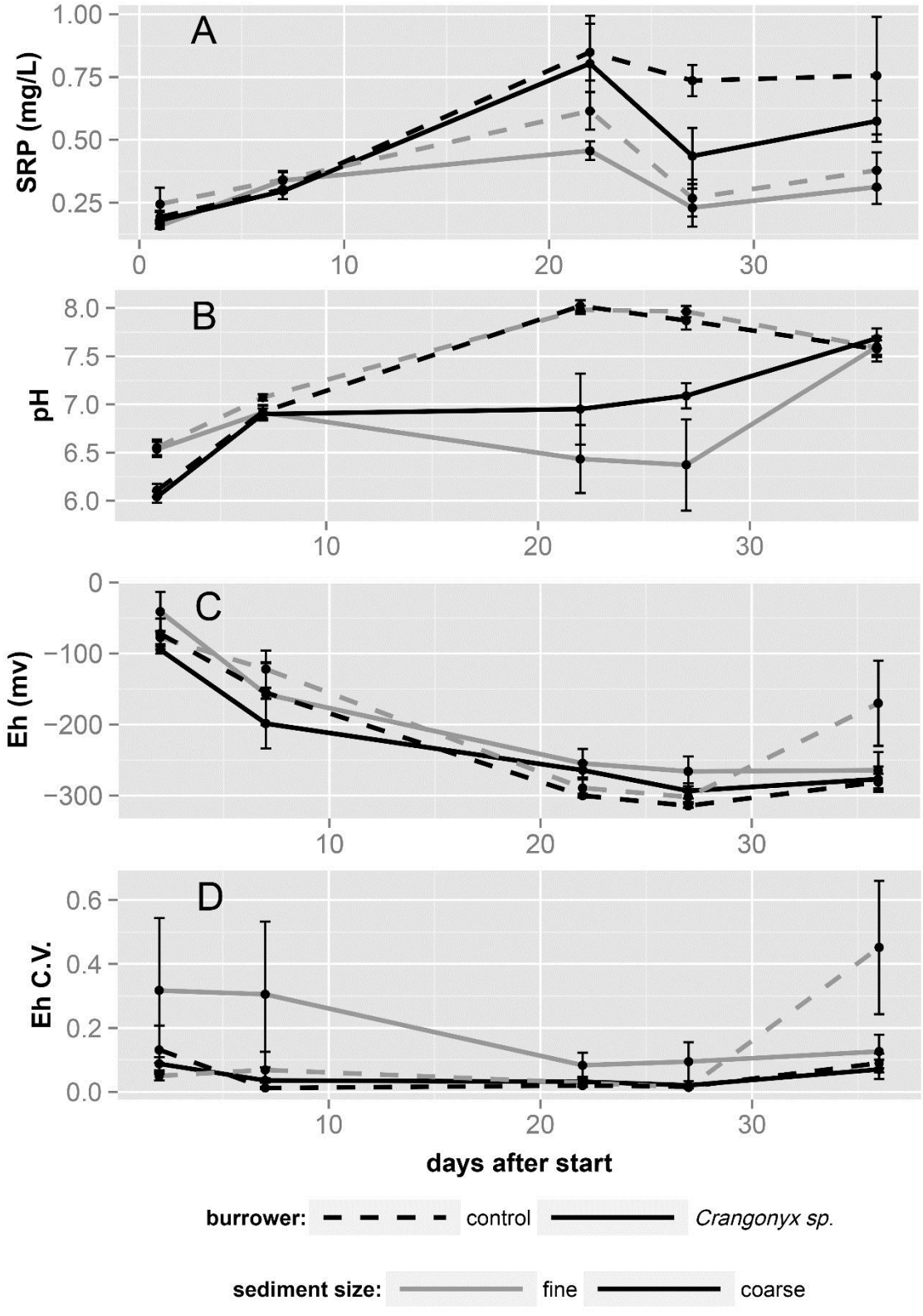
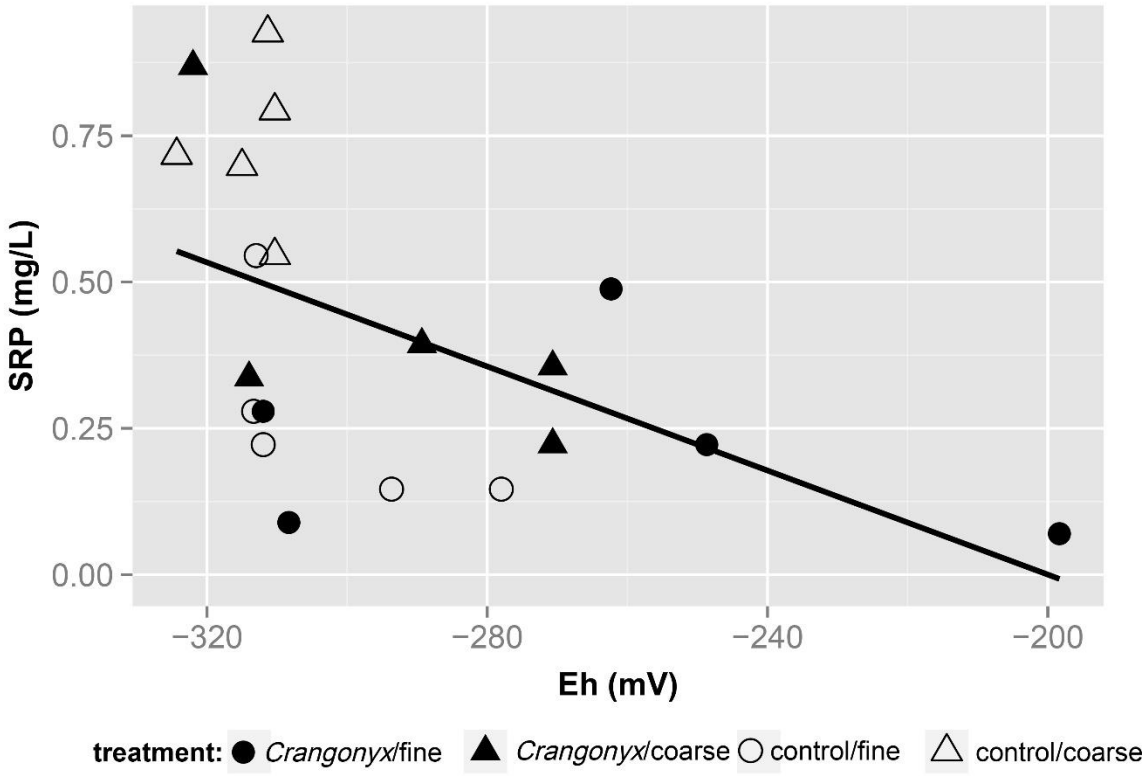


Figure 4.8 Relationship between mean redox potential measurement and SRP concentration after flushing microcosms with water containing 1.0 mg/L PO_4^{3-} (27 days after start). Line represents least-squares regression (R^2=0.27, P=0.018).



4. C. decorus *(gallery-diffusor)*

At 21 days after start, and 14 days of burrowing by *C. decorus* larvae, there was no effect of sediment size on SRP concentrations, and there was a significant effect of the burrower treatment (F=13.4015; df=1, 16; *P*=0.002), and a significant interaction between the two factors (F=6.501; df=1, 16; *P*=0.021) (Fig. 4.9a). Comparisons between burrower treatments within the two types of sediment show that microcosms with *C. decorus* had higher SRP concentrations than controls in microcosms with fine sediment (t=4.391; *P*<0.001). There was no difference in SRP concentration between microcosms with and without *C. decorus* with coarse sediment. There was not a significant relationship between SRP concentration and pH or redox potential. After flushing

microcosms with water containing 1.0 mg/L PO_4 at 23 days after start, microcosms with coarse sediment had higher SRP concentrations than microcosms with fine sediment (F=4.965; df=1, 16; *P*=0.041), and there was no effect of *C. decorus*. After incubating for 10 days, there were significant effects of sediment size (F=9.284; df=1, 16; *P*=0.008), *C. decorus* (F=16.505; df=1, 16; *P*<0.001), and their interaction (F=7.600; df=1, 16; *P*=0.014). Comparison of the simple-effects means shows that within microcosms with fine sediment, those with *C. decorus* larvae had lower SRP concentrations than controls (t=4.822; *P*<0.001), and within microcosms receiving *C. decorus* larvae, microcosms with coarse sediment had higher SRP concentrations than microcosms with fine sediment (t=4.104; *P*=0.002). There was not a significant relationship between SRP and redox potential, but there was a significant linear relationship between pH and SRP concentration at the final sample date. Microcosms with lower pH values had lower SRP concentrations (R^2=0.40; P=0.004) (Fig. 4.10). For the measurement at 33 days after start, *C. decorus* had a significant effect on redox potential (F= 6.928; df=1, 16; *P*=0.018), and the variation in redox potential within a microcosm (F= 7.612; df=1, 16; *P*=0.014). Microcosms with *C. decorus* larvae had higher redox potentials and also had higher variation between measurements within microcosms than controls (Fig 4.9c,d). There were also significant effects of burrowers (F=34.099; df=1, 16; *P*<<0.001), sediment (F=6.843; df=1, 16; *P*=0.019), and their interaction (F=4.808; df=1, 16; *P*=0.045) on pH at 33 days after start. For both levels of the sediment treatment, microcosms with *C. decorus* have lower pH than controls, and for burrower treatments only, microcosms with fine sediment have lower pH than coarse sediment (Fig. 4.9b).

Figure 4.9 Response variables measured from microcosms with C. decorus *(gallery-diffusor): (A) soluble reactive phosphorus concentrations, (B) pH, (C) redox potential, (D) coefficient of variation for replicate redox measures within a microcosm.*

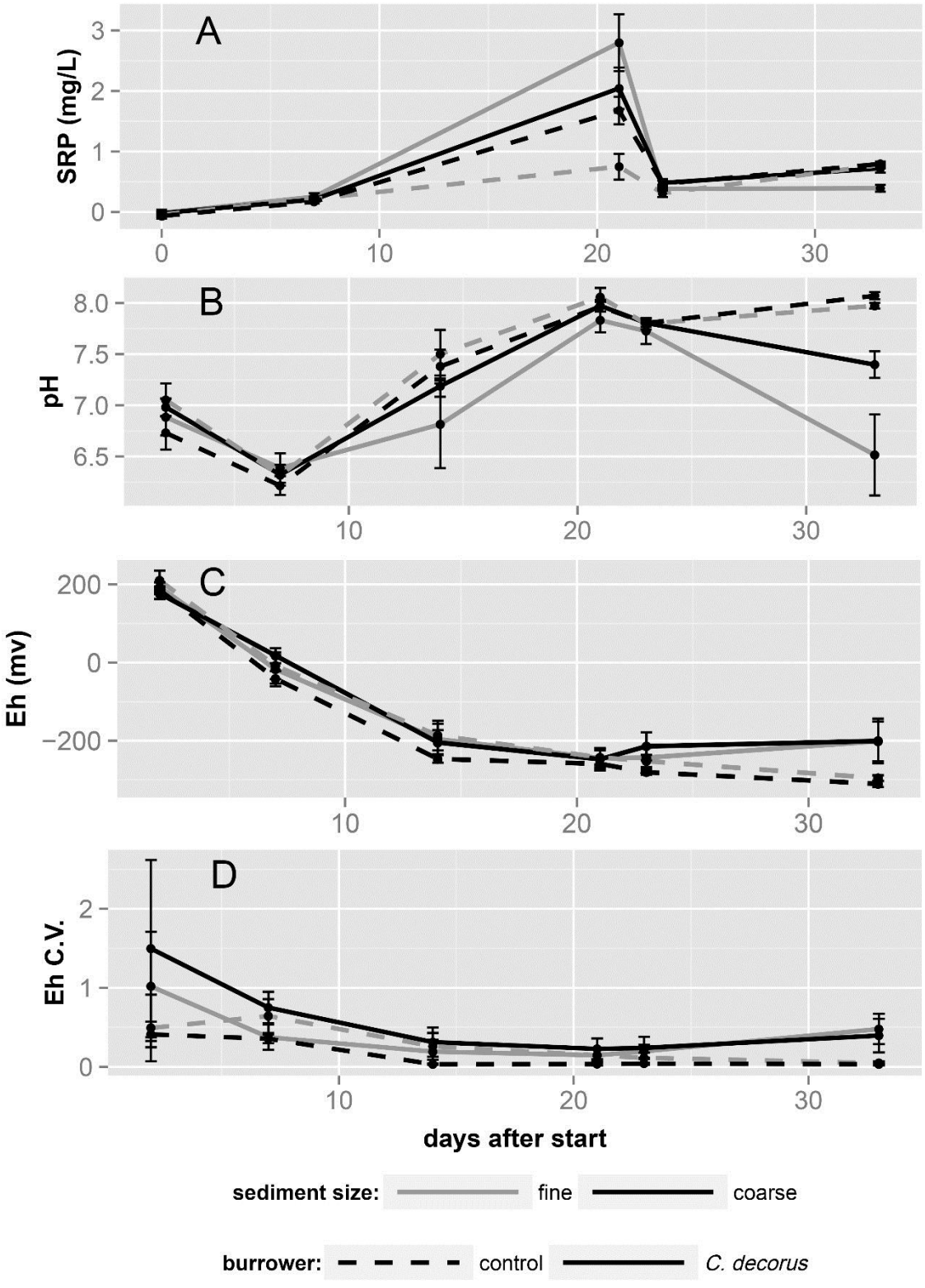
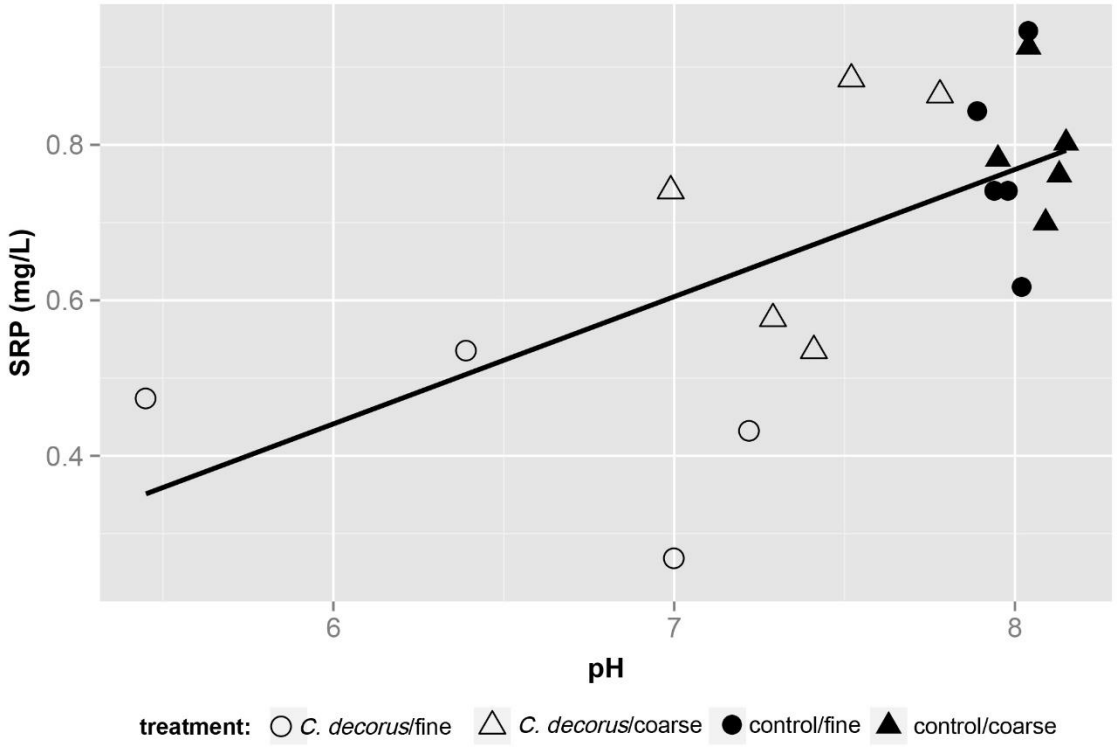


Figure 4.10 Linear relationship between SRP measured after a week of incubation following flush with 1mg/L PO_4 (33 days after start). Line represents least-squares regression (R^2=0.40, P=0.004).



Discussion

Bioturbation is an important ecological function of macroinvertebrates in aquatic ecosystems (Meysman et al. 2006). Understanding how different burrowing species alter rates of exchanges between subsurface sediments and surface water is important for understanding how aquatic ecosystems function. The objective of this study was to determine how four of the most common species of burrowing macroinvertebrates in Maryland drainage ditches affect the exchange of phosphorus between the sediment and surface water using microcosms. As a part of farming infrastructure, drainage ditches are subject to receiving high amounts of nutrients from fertilizers and manures applied to adjacent fields (Kleinman et al. 2007, Needelman et al. 2007a). Understanding the role of macroinvertebrates in the exchange of nutrients such as phosphorus between the sediment

and surface water of drainage ditches may lead to better management of drainage ditches to reduce delivery of excess nutrients to regional watersheds. From these experiments, I have shown that burrowing macroinvertebrates can alter dynamics of phosphorus exchange between sediment and surface water in ditches, resulting in different amounts of phosphorus available to be transported to local watersheds. The magnitude and direction of these effects depends both on the type of bioturbation produced by the burrower and conditions within the sediment and water (Table 4-2).

Table 4-2 Summary of differences measured in SRP concentration between the two levels of experimental factors (burrower and sediment size) across the four experiments for three sampling periods. Only differences that were statistically significant ($P<0.05$) are reported. Unless otherwise noted, differences represent magnitude of the main effect for that factor. "Type" refers to the bioturbation functional group into which that organism is classified. The measurements refer to (bioturbation) the first measurement taken after introduction of burrowers, (flush) the measurement taken immediately after flushing microcosms with water containing 1 mg/L PO_4, and (incubation) the final measurement after microcosms had incubated for an additional week.

			Difference in SRP (mg/L)	
Experiment	**Type**	**Measurement**	**Burrower bur. - ctrl.**	**Sediment coarse – fine**
I. templetoni	conveyor	bioturbation	-0.078	n.s.
I. templetoni	conveyor	flush	n.s.	n.s.
I. templetoni	conveyor	incubation	-0.270	0.364
L. hoffmeisteri	conveyor	bioturbation	n.s.	0.473
L. hoffmeisteri	conveyor	flush	0.276	0.758
L. hoffmeisteri	conveyor	incubation	-0.412	0.487
Crangonyx sp.	biodiffusor	bioturbation	n.s.	0.291
Crangonyx sp.	biodiffusor	flush	-0.169	0.337
Crangonyx sp.	biodiffusor	incubation	n.s.	0.320
C. decorus	gallery-diffusor	bioturbation	2.049 (fine)	n.s.
C. decorus	gallery-diffusor	flush	n.s.	0.127
C. decorus	gallery-diffusor	incubation	-0.386 (fine)	0.329 (burrower)

This study investigated different factors that could mediate the effect that burrowing macroinvertebrates have on the exchange of phosphorus in drainage ditches.

The first factor was the difference in bioturbation functional groups. The four experiments used four different species, which represent three different functional groups of burrowers. Differences in the effects produced by each species could be attributed to the differences in how they physically alter the sediment environment. The second factor under investigation was the habitat context in which bioturbation was happening. Specifically, I investigated the role of sediment structure in mediating the effect of bioturbation on phosphorus dynamics by manipulating the size to which sediment was crushed and sieved. The third factor under investigation was the effect of flow events through the system as compared to stagnant water. To make the results of this study specific to the drainage ditch environment, flows of water through the sediment profile were simulated. This allowed measurements to be taken not only of slow, longer term changes to the phosphorus concentration that result from bioturbation, but also changes in the ability of the sediment to remove excess phosphate from water through rapid sorption to surfaces. These different factors mediating the effect of bioturbation are discussed in reference to pH and the measured redox potential of the sediment. Other studies have measured changes in redox potential in sediments that result from burrowing by aquatic invertebrates, and altered redox chemistry could lead to differences in exchanges of phosphorus between the sediment and surface water (Hunting et al. 2012) Changes in pH may also affect the solubility of phosphorus adsorbed to iron and aluminum oxyhydroxides (Holdford and Patrick 1979).

The first measure of phosphorus after the introduction of burrowers simulated the effect of bioturbation on the equilibrium phosphorus concentration under stagnant conditions. As the microcosms were allowed to incubate for a week prior to the

introduction of burrowers, anaerobic reduction of iron in the sediment had likely released soluble phosphorus to the porewater, causing higher concentrations of phosphorus below the sediment than in the surface water (Young and Ross 2001). After the initial two weeks of burrowing, the small oligochaete worm, *I. templetoni* was the only species to produce lower SRP concentrations, while the larger oligochaete *L. hoffmeisteri* and *Crangonyx* had no effect, and *C. decorus* larvae caused an increase in the concentration of SRP in microcosms with fine sediment. The lack of a significant effect of *L. hoffmeisteri* after the initial incubation period may have been due to the system being overwhelmed with SRP at the time *L. hoffmeisteri* was introduced to the microcosms. Sediment used for this experiment produced SRP concentrations nearly 10 times higher than any of the other experiments at the time of introduction of burrowers. The difference in the direction of the effect between *I. templetoni* and *C. decorus* may be due to the different ways in which these two species burrow. As a deposit feeder, *I. templetoni* feeds directly on sediment particles, digesting microbial biofilms and other organic matter and egesting sediment pellets at the surface. Feeding directly upon the microbial biomass may have stimulated microbial production and uptake of available SRP, and the movement of sediment material from below-ground to the surface may have made more microbes exposed to available SRP in the surface water (Huang et al. 2010). This hypothesis is supported by redox measurements, which were significantly lower in microcosms with burrowers and fine sediment. Stimulation of microbial production through bioturbation could produce the lowered redox potentials as respiring microbes consume available terminal electron acceptors. In contrast, *C. decorus* larvae construct U-shaped burrows that they keep aerated as they move water through using peristaltic undulations of the

abdomen. This movement of surface water through burrows can increase the rate of exchange of porewater from the sediment to the surface, and can increase the rate of diffusion of SRP from sediment to the surface (Lewandowski et al. 2007). This hypothesis of increased exchange is supported by the effect of sediment size on SRP concentration in the two experiments where there was not a significant effect of bioturbation after the first incubation period. In the experiments with *L. hoffmeisteri* and *Crangonyx*, microcosms with fine sediment had lower SRP concentrations than microcosms with coarse sediment. Since there were no measured differences in the redox potentials for these two treatments, the difference is likely due to differences in diffusion rates of SRP from the sediment to the surface, and not different rates of SRP release from anaerobic reduction of iron oxides. *C. decorus* larvae produced increased surface area with their burrows and a constant movement of water through subsurface sediments, which allowed more movement of porewater SRP to surface water. This effect was only seen in fine sediment because coarse sediment already has higher rates of diffusion from sediment to the surface water through macropores produced by the non-uniform sediment aggregates.

Phosphorus measurements taken after flushing microcosms with water containing 1.0 mg/L PO_4 simulate discharges of water to ditches from adjacent fields as subsurface flow. Differences in phosphorus concentration between burrower and non-burrower treatments determine whether bioturbation alters the ability of the sediment to rapidly take up phosphorus added to the system through chemical or biological processes. Different species of burrowers produced different effects on SRP concentrations after flushing microcosms with water containing excess phosphorus. Burrowing by *Crangonyx*

caused lower SRP concentrations, while there was no effect of *I. templetoni* or *C. decorus* larvae, and *L. hoffmeisteri* caused an increase in the SRP concentration. The reduction in SRP concentration in treatments with *Crangonyx* may have been due to an increase in adsorption to iron oxide and oxyhydroxide surfaces. Microcosms with *Crangonyx* had significantly higher redox potentials than controls, and there was a significant and negative relationship between redox potential and SRP concentration across microcosms. Microcosms with *Crangonyx* also had significantly lower pH than controls, which would have decreased the solubility of phosphate-iron complexes. As biodiffusors, *Crangonyx* are constantly disrupting surface sediments, which may increase the availability of dissolved oxygen within the sediment and allow more iron oxides to remain stable. The increase in SRP concentrations produced by *L. hoffmeisteri* following the flush with high-phosphate water may have been a result of increased porosity of the sediment produced by larger burrows excavated by these larger-bodied worms. In contrast, the smaller burrows produced by *I. templetoni* may have been the reason there was not an effect seen in this experiment. Increased porosity would mean shorter residence time of the water moving through the sediment to the surface, and less interaction between the incoming water and sediment surfaces.

The final phosphorus measurement determined the effect of burrowers on longer-term phosphorus uptake of additions to the system. Processes that could produce slower uptake of phosphorus include precipitation of secondary phosphate minerals and incorporation into microbial biomass. The most consistent effect of bioturbation across the different species occurred at the final sampling point, after the second incubation period. *I. templetoni* and *L. hoffmeisteri* produced lower SRP concentrations across both

sediment treatments and *C. decorus* larvae produced lower SRP concentrations in microcosms with fine sediment, while *Crangonyx* had no effect. The lower SRP concentrations produced by the two oligochaete species are likely a result of their effect on microbial production. Redox chemistry may also play a role in the effect produced by *L. hoffmeisteri*, as redox potentials were significantly higher in microcosms with *L. hoffmeisteri* than controls, although there was not a significant relationship directly between SRP concentration and redox potential. The effect of *C. decorus* larvae is the reverse of what was measured after the first incubation period, where *C. decorus* increased the SRP concentration relative to controls. The reason for the reverse of effects may be due to differences in redox chemistry, as microcosms with *C. decorus* had higher redox potentials, and more variability between redox measurements than controls. The increased redox potential could result in more stable iron oxides to adsorb dissolved phosphorus, and the variability in redox potentials could benefit the microbial community, as the presence of both aerobic and anaerobic bacteria could increase efficiencies of assimilation of SRP into microbial biomass. Water pH was significantly lower in microcosms with *I. templetoni, L. hoffmeisteri*, and *C. decorus*, than controls, which could have resulted in decreased solubility of the adsorbed complexes of orthophosphate and newly precipitated iron oxyhydroxides in the surface waters of the microcosms.

In flooded, anaerobic soils and sediments such as drainage ditches, the availability of orthophosphates in solution is determined primarily by adsorption and desorption to oxidized iron and aluminum (Richardson 1985). The stability of iron oxides and oxyhydroxides is determined by the redox potential of the system, with reduction of ferric

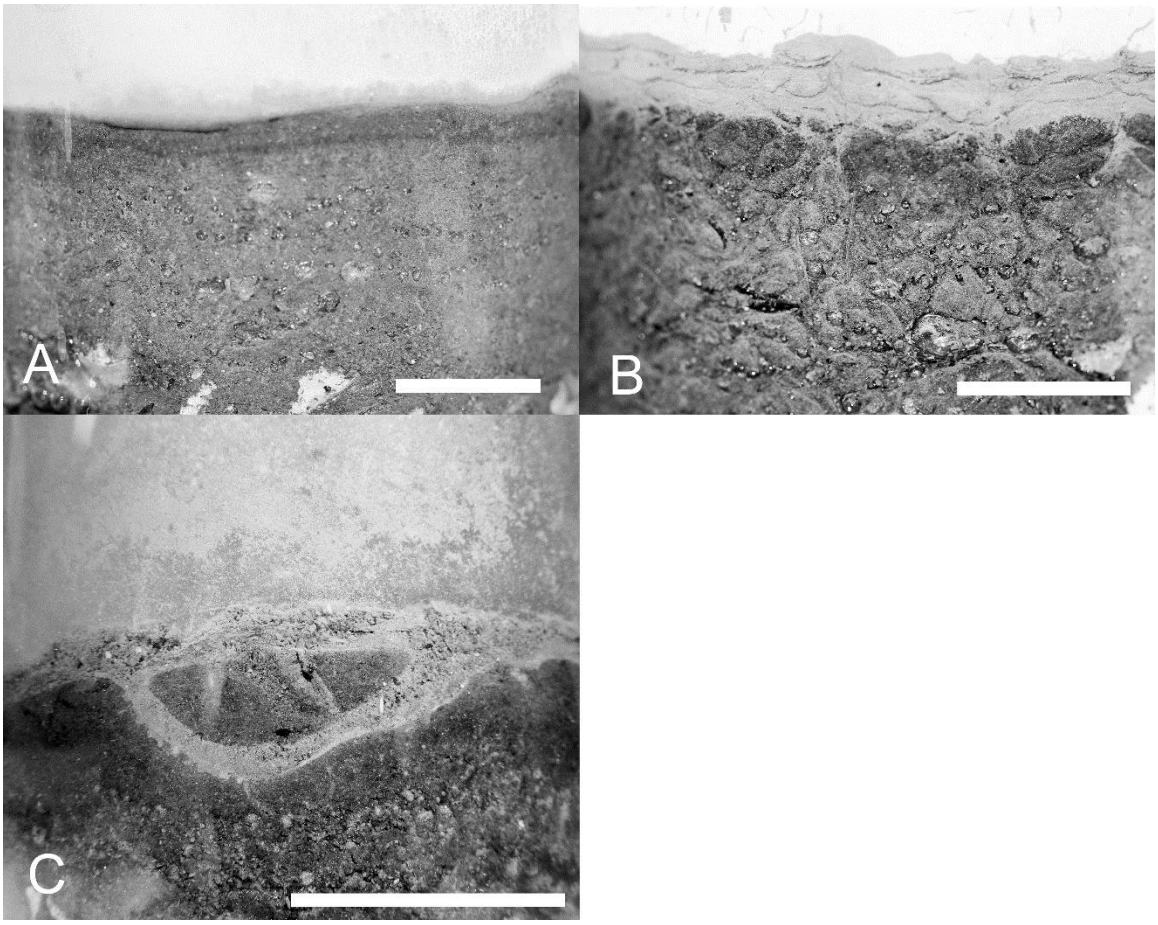
iron to soluble ferrous iron occurring at low redox potentials, coupled with the release of any adsorbed phosphates (Ponnamperuma 1972). In the microcosm experiments, iron was apparently reduced from sediments and translocated to the surface water, where it was then oxidized, which was evident in microcosms as a rusty, orange coating of the sediment surface and sides of the beakers. The strength of adsorption of orthophosphate to iron and aluminum oxide surfaces is determined by the pH of the system, with adsorption being favored at lower pH (Holford and Patrick 1979). The precipitation of iron and aluminum phosphates is also favored at lower pH. In measurements where burrowers had a significant effect on phosphorus concentrations, there was also often a significant effect of burrowers on either pH or redox potential. For all four species, measurements where burrowers produced lower phosphorus concentrations relative to controls were coupled with significant effects of burrowers on pH, with lower pH values being measured in burrower treatments. For three of those measurements, there was also a significant effect of burrowers on redox potential, with higher redox potentials being measured in burrower treatments. Burrowing species can alter redox potentials in the sediment by constructing burrows that allow oxygen-rich surface water to move deeper into sediment layers. Higher redox potentials would also be measured in microcosms as a result of increased proton concentration with lower pH measures. Burrowing may have lowered the pH in the system by exposing iron monosulfides, which are common in ditches on Maryland's Eastern Shore, to the surface, which produce acidity upon oxidation (Needelman et al. 2007b). Iron monosulfides were apparent in microcosms at the completion of the experiment as a black coating on the surface of portions of the beaker and redox electrodes below the sediment surface. These black coatings reacted

with hydrogen peroxide to produce the rotten-egg smell of hydrogen sulfide gas and turned a rusty, orange color, which identified them as iron monosulfides.

L. hoffmeisteri and *C. decorus* each caused an increase in phosphorus concentration relative to controls in one measurement. This suggests that in some cases, large deposit-feeding oligochaetes and gallery-diffusing fly larvae may contribute significantly to fluxes of SRP from sediment porewater to surface water. These effects were not measured in conjunction with changes to sediment or water chemistry, and therefore are likely due to differences in transport of phosphorus from sediment porewater to the surface that resulted from physical changes to the sediment, rather than changes to chemical immobilization or microbial uptake of phosphorus (Fig. 4.11). The effect of the conveyor seems to be mediated by the flow of water through the system. Burrows created by *L. hoffmeisteri* may have created preferential flow paths through the sediment layer (Fig. 4.11b), which allowed more dissolved phosphates to be transported to the surface water without interacting with surfaces within the sediment that could immobilize phosphorus. After the simulated flow event, *L. hoffmeisteri* reduced phosphorus concentrations relative to controls under stagnant water conditions. The effect of gallery diffusors seems to be mediated by the relative concentration of SRP in porewater compared to surface water. Microcosms were allowed to develop anaerobic conditions over a week of flooding with no disturbance of the sediment, and water pumped through U-shaped burrows by *C. decorus* larvae essentially caused a mixing of sediment porewater and surface water (Fig. 4.11c). Drainage ditch sediments can contain high amounts of exchangeable phosphorus, and are not often in equilibrium with the surface water (Nguyen and Sukias 2002). This can result in sediment porewater having

concentrations of SRP that are up to two orders of magnitude higher than the surface water (Hill and Robinson 2012). Soil phosphorus concentrations can also be highly variable within a ditch, which may make it possible for biodiffusors like *C. decorus* to enhance and reduce the movement of SRP within a ditch simultaneously (Vaughan et al. 2007).

Figure 4.11 Examples of physical changes caused to sediment by different burrowers. (A) Control microcosm with no disturbance, (B) burrows and sediment relocation caused by L. hoffmeisteri, *and (C) U-shaped burrow of* C. decorus. *All images show fine sediment treatment. Scale bars represent 10 mm.*



This study demonstrates the importance of sediment structure in the dynamics of phosphorus movement from sediment to overlying water. With only two exceptions, the measurements of SRP were higher in microcosms with coarse sediment than fine

sediment. This suggests that diffusion of SRP from sediment porewater to the surface occurs faster in drainage ditches that have more aggregated structure in the sediment. The aggregated structure may produce macropores that act as preferential flow paths for water during flow events. This could enable faster rates of diffusion from the sediment to the water, and could result in less interaction between incoming water and sediment surfaces that could adsorb phosphorus. The clumped structure of sediment could also decrease the ability of porewater to interact with the inside of aggregates, and effectively reduces the surface area in contact with the porewater. Microbial biofilms occur on surfaces, and a reduction in the effective surface area could reduce the ability of microbes to take up SRP. This suggests that small, primary ditches that tend to have developed a granular structure from cycles of drying and wetting may release SRP at higher rates than large, collection ditches that tend to have single-grained sediments.

One current technique for managing drainage ditches to reduce the delivery of pollutants to receiving waters is to install flow-control devices that retain water within ditches during periods when it is not necessary to have full drainage. Increasing the duration of inundation in drainage ditches may improve conditions for retention of phosphorus by the sediment through sedimentation, and the production of flocculent iron oxyhydroxides to adsorb dissolved phosphorus (Usborne et al. 2013, Maynard et al. 2011). Maintaining standing water in ditches throughout the year may also reduce the amount of SRP released to surface water by preventing the formation of aggregated structure within the sediment profile.

As a microcosm study, the results from these experiments cannot be simply extrapolated to the field scale. There are many more factors at play in ditches than were

modelled in the microcosms that may further mediate any effect of bioturbation on phosphorus dynamics. The experiments were maintained at a constant temperature, while ditches may experience annual fluctuations in temperature from 0-30°C. The species used in these experiments are not found in isolation in nature, and interactions between one another and many other species present in ditches may alter their effect on phosphorus dynamics (Leslie et al. 2012, Ch. 3). Plants were completely excluded from the experiments, but likely play an important role in taking up SRP. The microcosms may have allowed unusually long residence times for the water to interact with sediment, but this condition may be realistic under conditions of low flow or impoundment with flow-control structures or weirs. Field manipulation of burrowing communities and measurement of their effects would be logistically difficult, but is a logical next step in fully understanding the nature of bioturbation in drainage ditches.

Bioturbation by the different species of burrowers had significant effects on movements of phosphorus to the surface water, and these effects differed between functional groups. Across all four experiments, bioturbation resulted in lower SRP concentrations in at least one of the three water measurements. Current management practices for drainage ditches focus on enhancing the ability of ditches to reduce the load of nutrients and sediment delivered to receiving waters as a way of preventing eutrophication of aquatic habitats downstream. Few studies have focused on the macroinvertebrate communities within ditches, and those studies have not considered the direct role that macroinvertebrates play in altering water quality. This study has shown that burrowing aquatic invertebrates can significantly increase or decrease the concentration of SRP in surface waters, depending on the type of bioturbation and the

relative availability of phosphorus in the ditch habitat. The effect of increasing diversity among burrowers on SRP concentrations in surface water is as yet unknown. The mechanisms producing changes in phosphorus concentrations are likely varied, as burrowers alter the microbial community, advection of solutes, and redox chemistry simultaneously, the interactive effects of multiple species may have unpredictable effects.

If different functional groups of burrowers affect different factors that control phosphorus dynamics in ditch sediments, this makes it possible for there to be non-additive effects of combined bioturbation activities of multiple species. There could be greater than additive effects from combined bioturbation affecting translocation of sediment materials and associated microbes (conveyors) and the aeration of surface sediments (biodiffusors). There may also be biotic interactions between different burrowers as they compete for limited space within ditch sediments, which could result in altered foraging behaviors, and changes in the effects of bioturbation on phosphorus dynamics. Future studies should address this interplay between functional groups, to determine how these interactions affect ecosystem functions.

Chapter 5 - Conclusions: The structure and function of ditch benthic invertebrate communities

Agriculture remains the land-use category responsible for impairment of the most freshwater aquatic and coastal marine habitats in the United States. In order to maintain high agricultural productivity while protecting natural water resources, innovative solutions will have to be found to prevent the movement of pollutants such as excess nutrients from agricultural fields into local waterways. Recently, drainage ditches have been recognized as a location where mitigation strategies can be targeted to increase the quality of water draining from agricultural lands into local watersheds. These mitigation strategies have focused on augmenting biogeochemical transformations of agrochemicals in ditch water as a way of reducing transport of pollutants to receiving waters. This research has found that sediments and developing soils within drainage ditches have the capacity to remove excess nitrates, immobilize phosphates, and break down pesticides, while maintaining drainage functions within ditches. For agricultural lands under artificial drainage, ditches may provide an important resource for managing the impacts of agriculture on regional water quality.

Studies of nutrient transformations in drainage ditches have been somewhat limited in that, aside from the microbial community, they have neglected to quantify impacts from the biological communities of drainage ditches. In natural streams, the macroinvertebrate community plays important roles in cycling of nutrients and decomposition of organic matter. Although drainage ditches are far from natural headwaters, they do provide habitat for aquatic macroinvertebrates, which may in turn have a significant impact on biogeochemical cycles within ditches. One function of macroinvertebrates that may play an important role in nutrient cycling is bioturbation.

Many studies from lakes and coastal marine habitats have shown that burrowing by benthic macroinvertebrates can have effects such as altered redox chemistry of sediments, mixing of porewater and surface water, and redistribution of materials at the sediment-water interface. These changes can, in turn affect microbial diversity and productivity, which results in different rates of nutrient transformations.

The overall goal of this research was to determine the biodiversity of aquatic macroinvertebrates living in agricultural drainage ditches on the Eastern Shore of Maryland, and to determine what role they play in ecosystem-scale functions occurring in ditches. To complete this research, I started with general surveys of aquatic invertebrates, with no prior knowledge of the community composition or structure along environmental gradients (Chapter 2). From this study, I learned that the majority of the community of macroinvertebrates in ditches are associated with the sediment, and that more detailed knowledge of community composition and density would have to target that habitat specifically. A second, more intensive survey was conducted to determine the composition of sediment-dwelling macroinvertebrates present in field (intermittent) and collection (perennial) ditches, and to determine the composition of functional groups represented in each (Chapter 3). From this study, I determined that macroinvertebrates in ditches represent different types of bioturbators, and that macroinvertebrate effects on ecosystem functions would likely be the result of bioturbation by macroinvertebrates. Finally, microcosm experiments were used to determine specific effects that different types of bioturbation have on nutrient dynamics in ditch sediment (Chapter 4). From this study, I learned that different types of burrowers have different effects on phosphorus dynamics, depending on the environmental context. Under some conditions, burrowing

invertebrates may increase rates of phosphorus release from sediment to surface water, but all representative burrowers decreased phosphorus concentrations in surface water relative to controls in at least one measurement. Confirmation of this effect outside of a microcosm is needed with in-ditch experiments, but the results of these studies suggest that there is potential for aquatic macroinvertebrates to play a significant role in regulating phosphorus dynamics in drainage ditches.

Chapter 2: Environmental Factors Structuring Benthic Macroinvertebrate Communities of Agricultural Ditches in Maryland

There have been relatively few studies of aquatic invertebrate communities of agricultural drainage ditches as compared to natural aquatic habitats. One reason for the lack of study in the United States may be that ditches are not regulated as natural bodies of water, and therefore have not been subject to biomonitoring. Other studies from around the world have found that ditches are capable of sustaining communities of aquatic macroinvertebrates, with some studies highlighting the conservation value of aquatic habitats provided by drainage ditches. In order to determine the functional roles of macroinvertebrates in drainage ditches, the structure of the community must first be determined. Therefore, for my first chapter, I performed surveys to determine what taxa of macroinvertebrates inhabit drainage ditches on Maryland's Eastern Shore, and how those communities respond to environmental characteristics measured within those ditches.

For this study, I collected macroinvertebrates and environmental data from 29 drainage ditches across three counties on Maryland's Eastern Shore to determine the composition of macroinvertebrate communities in drainage ditches, and their relationship

with environmental gradients. The environmental variables that were measured were chosen to take into account habitat requirements of macroinvertebrates (i.e. pH, dissolved oxygen, and conductivity), as well as variables related to nutrient cycling within ditches (i.e. water and soil nutrients, soil redox potential), and physical habitat characteristics (detritus cover, flow velocity, ditch size). Analysis of community composition across ditches showed that there are differences in the macroinvertebrate community across ditches, and that there is not a single homogeneous community of invertebrates. Multivariate analysis of environmental factors shows that community composition is related primarily to physical habitat characteristics and not to water quality measures.

Several previous studies of drainage ditch communities have highlighted the presence of a few rare taxa, or the presence of high taxonomic diversity at landscape scales as evidence of the biodiversity value of drainage ditches. Management implications from these studies suggest minimal disturbance of the ditch habitat to maintain a more stable environment that is more suitable for these rare taxa. Other studies have tried to superimpose bioassessment strategies developed for natural streams onto drainage ditches in an attempt to develop metrics of ditch health. These attempts seem to have had minimal success, since classical bioassessment requires a "least disturbed" reference site against which to compare ditches, and as man-made structures, there may be no "pristine" ditches in existence to make these comparisons. The merit of these conservation strategies for maintaining drainage ditches may vary as a function of the available natural habitat in the landscape. The fewer remaining natural streams, ponds, and wetlands, the more valuable drainage ditches may become as habitat that conserves aquatic macroinvertebrate diversity. From my initial survey of drainage ditch

communities, I found 90 taxa of macroinvertebrates, 82 of which were also found in natural streams in the same area by the Maryland Biological Stream Survey (MBSS), and none of which are listed as threatened or endangered.

Many of the design qualities that increase the drainage functions of ditches also make them poor habitats for aquatic life. Although drainage ditches do have the potential for supporting a wide variety of aquatic life, these are highly modified and highly disturbed waterways that serve a primary function as agricultural infrastructure, and only a secondary role as aquatic habitat. As a result, even in cases where high species richness has been documented in drainage ditches, the majority of the community is represented by generalist, tolerant taxa, such as chironomid fly larvae and oligochaete worms, which were numerically dominant in the communities measured in this study. Under classical bioassessment protocols, the lack of taxonomic and functional feeding group diversity would indicate a loss of functions for these aquatic habitats. However, I would argue that those assessments have been developed for natural stream habitats, and that they do not apply to drainage ditches. My research has focused on determining the functions of the existing populations of common, generalist species in drainage ditches, rather than cataloging natural stream functions that do not exist there.

Chapter 3: Patterns of burrowing invertebrate communities in small and large agricultural drainage ditches

Bioturbation by burrowing benthic invertebrates is a function of aquatic macroinvertebrates that can have ecosystem-level effects on the transformation and transportation of nutrients. On the Eastern Shore of Maryland, much of the water enters drainage ditches through subsurface flow paths, and therefore must interact with the

sediment before being discharged to the surface. Sharp chemical gradients that occur across the sediment-water interface make this a site of intense biogeochemical transformations, which can determine whether nutrients get delivered to the surface water, or remain sequestered within the sediment. From surveys performed in Chapter 2, I found that a large proportion of the community of macroinvertebrates in ditches live on or in the sediment, and therefore could be causing bioturbation in ditch sediments. To quantify the potential for aquatic macroinvertebrates in ditches to produce bioturbation of ditch sediments, I sampled sediment-dwelling macroinvertebrates, and used functional groups to determine potential roles within ditches.

To sample sediment-dwelling macroinvertebrates directly, I collected sediment cores, rather than D-net sweeps, which also collect organisms from the water column and attached to plants. My sampling was divided between small (primary) ditches and large (collection) ditches, since ditch size was found to be a significant factor influencing the composition of macroinvertebrate communities in Chapter 2. Sampling was repeated monthly to determine whether there were any seasonal changes in the burrowing community, and all samples of macroinvertebrates were sorted and identified in entirety, which gave quantitative measures of abundance across all sites. Results from this study showed that there are significant differences in community composition between small and large ditches, but similar functional groups are represented by different taxa in each habitat type. Functional feeding group diversity is relatively low, with the majority of all macroinvertebrates belonging to the collector-gatherer (detritivore) functional group. Functional groups of bioturbation are well represented within the sediment-dwelling

community, and may represent the most important function of aquatic macroinvertebrates in ditch ecosystems.

According to my surveys of burrowing invertebrate communities, bioturbation is ubiquitous among large and small ditches, and across seasons. Large and small ditches have different community composition of macroinvertebrate species, but have similar representatives of different bioturbation functional groups. Burrowing communities in field and collection ditches do not differ in density, species richness, or across seasons. Bioturbation is a function of the macroinvertebrate community occurring constantly, across all drainage ditches. Bioturbation could affect rates of nutrient cycling (for nitrogen and phosphorus), as well as decomposition rates of organic matter, the transport of mineral sediment, and rates of recruitment of dormant stages in the sediment to the ditch plant, animal, and microbial communities. The specific effects that the burrowing community will have on ditch functions will likely vary as a function of the environmental context in which those species are burrowing. There will likely be differences in the effect between large and small ditches, since the physical habitat characteristics between those two habitats differs as a function of seasonal drying that occurs in small ditches. There will also likely be differences in effects between ditches with faster flow velocities than ditches with slower flow velocities. Previous work on effects of bioturbation has shown that different effects can occur in systems that are advection dominated (fast-flow) compared to diffusion dominated (slow-flow).

Chapter 4: Burrowing macroinvertebrates alter phosphorus dynamics in drainage ditch sediments

Phosphorus transport in Maryland watersheds is a primary concern for the protection and restoration of the Chesapeake Bay, which suffers from the effects of eutrophication caused in part by increased phosphorus loading from agricultural runoff. Several studies have found that ditch sediments can take up phosphorus from water draining agricultural fields, but this effect is not always consistent, and may depend on many factors including mineralogy, hydrology, and history of human activities on the land. Bioturbation by benthic invertebrates has been shown to affect phosphorus exchanges between sediment and water in river, lake, and coastal marine systems. The presence of a large and ubiquitous community of burrowing invertebrates across drainage ditches suggests that bioturbation may have a similar effect in drainage ditch sediments. The objective of Chapter 4 was to determine the effect of different burrowing species on phosphorus exchanges between sediment and water using microcosms to simulate conditions within drainage ditches.

Microcosms were constructed to simulate the difference in structure between small and large ditch sediments, as well as different flow conditions that occur within drainage ditches on Maryland's Eastern Shore. The physical structure of the sediment was manipulated to recreate the aggregated structure found in primary ditches and the single-grained structure found in collection ditches. Four species of burrowers were chosen to represent some of the most abundant species found from surveys in Chapter 3, while also representing different functional bioturbation groups. Water conditions were manipulated to represent (1) a stagnant condition, (2) short-term uptake during a discharge event, and (3) long-term uptake following a flow event. Results showed different effects of different species under different environmental conditions, however

all species reduced soluble, reactive phosphorus concentrations in the water relative to controls under at least one set of environmental conditions. Two species were also found to increase the concentration of SRP in the surface water relative to controls under other environmental conditions.

These experiments showed that different functional groups of burrowers have different effects on phosphorus dynamics, but also that those effects depend upon the environmental context within the experimental microcosm. The different effects of different types of burrowers reflect the different physical changes they bring about to the sediment habitat. Some burrowers actively mix sediments by feeding and depth and depositing sediment at the surface, others randomly scatter surface sediments, and others live within tunnels that they keep aerated by moving surface water through their burrows. These different physical processes interact differently with different types of sediment environments to create different measurable effects on phosphorus dynamics. Concentration gradients that exist between sediment porewater and surface water may also determine the scale and direction of the effects of different burrowers on phosphorus dynamics. Taken singly, it is difficult to generalize the effect of burrowing macroinvertebrates on phosphorus dynamics across different combinations of sediment and water treatments. If one were to take into account that these different burrowers all occur together in ditches, predicting the net effect on phosphorus dynamics would be even more difficult. One generalization that could be made across all taxa used for these experiments is that under at least some conditions, burrowers decrease surface phosphorus concentrations relative to controls. This does not necessarily occur under the same environmental conditions for all burrowers, but the mechanisms causing this change

seem to be related to the stability of iron oxides through altered redox chemistry, and the stability of iron and aluminum-phosphorus bonds through decreased pH.

Identifying the effects of bioturbation in the laboratory is an important first step in quantifying the results of that effect at the ecosystem scale. To fully understand the real-world effect of burrowing species on phosphorus dynamics in ditches, field experiments will have to be conducted in the future to measure the effect of the burrowing community on nutrient dynamics. The effects measured from microcosm experiments occurred under idealized conditions, where sediment composition was homogenized and uniform across experimental units, interspecies interactions were eliminated, and the effect of flows within ditches was isolated to a single measurement, rather than incorporated throughout the experiment. Real-world conditions within ditches are very different from the beakers held in the laboratory, and the effects that would be measured are also likely to be very different. Once real-world effects of burrowing communities can be quantified across gradients of environmental conditions, it may be possible to include beneficial effects of burrowing invertebrate communities in drainage management practices targeted at reducing nutrient exports to streams and rivers.

The results of this research are specific to drainage ditches on Maryland's Eastern Shore, which may not apply to drainage networks in other parts of the world, or even within the continental United States. The underlying geology of the different regions may determine the relative effect of bioturbation on biogeochemical processes in ditch sediments. Maryland's Eastern Shore is a part of the Delmarva Peninsula, which is a land mass formed from the accumulation of marine sediments. Ditches draining wet prairies in the Midwest or swamps of the Mississippi delta have sediments derived from different

mineralogy, and likely differ in chemical properties, and may alter the effect that bioturbation has on nutrient dynamics and other biogeochemical processes.

Conclusions

My research has identified and described the aquatic macroinvertebrate community from an under-studied habitat on Maryland's Eastern Shore, and has identified the primary role of this community in the functioning of that habitat. Although drainage ditches are superficially similar to natural streams, they differ substantially in structure and function, and the macroinvertebrate community is no exception. It would be incorrect to describe the communities of drainage ditches as low quality or impaired streams, just as it would be incorrect to make the same assessment of a pond or wetland habitat. Drainage ditches are man-made structures, and as such are unique with regards to their intended use and functions, and should not necessarily have standards ascribed to them from their nearest natural counterparts. Instead, by design, the primary function of drainage ditches is to lower the water table to produce arable fields, and the secondary function, by necessity, is to reduce the amounts of pollutants entering adjacent waterways from agricultural fields. Maintaining biological integrity of drainage ditches means maintaining a community of organisms that promotes these functions within the ditch habitat.

Bioturbation is a function of the aquatic macroinvertebrate community that could have effects on functions of drainage ditches. My experiments have shown that burrowing invertebrates from drainage ditches can alter phosphorus dynamics between sediment and the overlying water, which determines whether phosphorus is contained within the ditch or is transported downstream to the local watershed. Many other

chemicals besides phosphorus are exchanged between sediment and water in ditches, and bioturbation could have different effects on movements of those chemicals. For example, bioturbation could affect rates of denitrification by anaerobic bacteria, or the exchange of heavy metals through movements of sediment or bioaccumulation in deposit feeding organisms. Understanding chemical reactions occurring in drainage ditches is important for determining the fate of agriculturally derived pollutants, and understanding the role of bioturbation in regulating these transformations is necessary for a full understanding of the processes controlling the quality of water draining from agricultural lands.

References

74th United States Congress. 1936. Soil Conservation and Domestic Allotment Act.

Adámek, Z., and B. Maršálek. 2013. Bioturbation of sediments by benthic macroinvertebrates and fish and its implication for pond ecosystems: a review. Aquaculture International 21:1–17.

Alexander, R. B., E. W. Boyer, R. A. Smith, G. E. Schwartz, and R. B. Moore. 2007. The role of headwater streams in downstream water quality. Journal of the American Water Resources Association 43:41–59.

Alexander, R. B., R. A. Smith, and G. E. Schwartz. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature 403:758–761.

Allan, J. D., and M. M. Castillo. 2007. Stream ecology: Structure and function of running waters. 2nd edition. Springer, Dordrecht.

Angeler, D. G., S. Sánchez-Carrillo, G. García, and M. Alvarez-Cobelas. 2001. The influence of *Procambarus clarkii* (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. Hydrobiologia 464:89–98.

Armitage, P. D., K. Szoszkiewicz, J. H. Blackburn, and I. Nesbitt. 2003. Ditch communities: a major contributor to floodplain biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems 13:165–185.

Axt, J. R., and M. R. Walbridge. 1999. Phosphate removal capacity of palustrine forested wetlands and adjacent uplands in Virginia. Soil Science Society of America Journal 63:1019–1031.

Balvanera, P., A. B. Pfisterer, N. Buchmann, Jing-Shen He, T. Nakashizuka, D. Raffaelli, and B. Schmid. 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters 9:1146–1156.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish - second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water, Washington, D.C.

Bell, W. H., and P. Favero. 2000. Moving water: A report to the Chesapeake Bay Cabinet by the Public Drainage Task Force. Maryland Department of Natural Resources.

Blann, K. L., J. L. Anderson, G. R. Sands, and B. Vondracek. 2009. Effects of agricultural drainage on aquatic ecosystems: A review. Critical Reviews in Environmental Science and Technology 39:909–1001.

Boesch, D. F., R. B. Brinsfield, and R. E. Magnien. 2001. Chesapeake Bay eutrophication: Scientific understanding, ecosystem restoration, and challenges for agriculture. Journal of Environmental Quality 30:303–20.

Brooks, R. T. 2000. Annual and seasonal variation and the effects of hydroperiod on benthic macroinvertebrates of seasonal forest ("vernal") ponds in central Massachusetts, USA. Wetlands 20:707–715.

Bruland, G. L., and C. J. Richardson. 2006. An assessment of the phosphorus retention capacity of wetlands in the Painter Creek watershed, Minnesota, USA. Water, Air, & Soil Pollution 171:169–184.

Buchanan, C., K. Foreman, J. Johnson, and A. Griggs. 2011. Development of a basin-wide benthic index of biotic integrity for non-tidal streams and wadeable rivers in the Chesapeake Bay watershed: Final report to the Chesapeake Bay Program Non-Tidal Water Quality Workgroup. Interstate Commission on the Potomac River Basin.

Cardinale, B. J., and M. A. Palmer. 2002. Disturbance moderates biodiversity–ecosystem function relationships: Experimental evidence from caddisflies in stream mesocosms. Ecology 83:1915–1927.

Cardinale, B. J., M. A. Palmer, and S. L. Collins. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415:426–429.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological Applications 8:559–568.

Chacon, N., S. Flores, and A. Gonzales. 2005. Implications of iron solubilization on soil phosphorus release in seasonally flooded forests of the lower Orinoco River, Venezuela. Soil Biology and Biochemistry 38:1494–1499.

Chaffin, J. D., and D. D. Kane. 2010. Burrowing mayfly (Ephemeroptera: Ephemeridae: *Hexagenia* spp.) bioturbation and bioirrigation: A source of internal phosphorus loading in Lake Erie. Journal of Great Lakes Research 36:57–63.

Charbonneau, P., and L. Hare. 1998. Burrowing behavior and biogenic structures of mud-dwelling insects. Journal of the North American Benthological Society 17:239-249.

Clarke, A., R. Mac Nally, N. Bond, and P. S. Lake. 2008. Macroinvertebrate diversity in headwater streams: A review. Freshwater Biology 53:1707–1721.

Clarke, A., R. Mac Nally, N. R. Bond, and P. S. Lake. 2010. Conserving macroinvertebrate diversity in headwater streams: the importance of knowing the relative contributions of α and β diversity. Diversity and Distributions 16:725–736.

Colvin, R., G. R. Giannico, J. Li, K. L. Boyer, and W. J. Gerth. 2009. Fish use of intermittent watercourses draining agricultural lands in the Upper Willamette River Valley, Oregon. Transactions of the American Fisheries Society 138:1302–1313.

Conley, D. J., H. W. Paerl, R. W. Howarth, D. F. Boesch, S. P. Seitzinger, K. E. Havens, C. Lancelot, and G. E. Likens. 2009. Controlling eutrophication: Nitrogen and phosphorus. Science 323:1014–5.

Cooper, C. M. 1993. Biological effects of agriculturally derived surface water pollutants on aquatic systems—A review. Journal of Environmental Quality 22:402–408.

Cooper, C. M., M. T. Moore, E. R. Bennett, S. Smith Jr., J. L. Farris, C. D. Milam, and F. D. Shields Jr. 2004. Innovative uses of vegetated drainage ditches for reducing agricultural runoff. Water Science and Technology 49:117–123.

Covich, A. P., M. C. Austen, F. Bärlocher, E. Chauvet, B. J. Cardinale, C. L. Biles, P. Inchausti, O. Dangles, M. Solan, M. O. Gessner, B. Statzner, and B. Moss. 2004. The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. BioScience 54:767–775.

Covich, A. P., M. A. Palmer, and T. A. Crowl. 1999. The role of benthic invertebrate species in freshwater ecosystems. BioScience 49:119-127.

Covich, A. P., and J. H. Thorp. 2001. Introduction to the subphylum Crustacea. Pages 777–810 *in* J. H. Thorp and A. P. Covich, editors. Ecology and Classification of North American Freshwater Invertebrates. Second edition. Academic, New York, NY.

Creed, R. P., A. Taylor, and J. R. Pflaum. 2010. Bioturbation by a dominant detritivore in a headwater stream: Litter excavation and effects on community structure. Oikos 119:1870–1876.

Crowl, T. A., W. H. McDowell, A. P. Covich, and S. L. Johnson. 2001. Freshwater shrimp effects on detrital processing and nutrients in a tropical headwater stream. Ecology 82:775–783.

Cummins, K. W. 1974. Structure and function of stream ecosystems. BioScience 24:631–641.

Cummins, K. W., and M. J. Klug. 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics 10:147–172.

Davies, B., J. Biggs, P. Williams, M. Whitfield, P. Nicolet, D. Sear, S. Bray, and S. Maund. 2008. Comparative biodiversity of aquatic habitats in the European agricultural landscape. Agriculture Ecosystems and Environment 125:1–8.

Davies, S. P., and S. K. Jackson. 2006. The biological condition gradient: A descriptive model for interpreting change in aquatic ecosystems. Ecological Applications 16:1251–1266.

Davis, S., S. W. Golladay, G. Vellidis, and C. M. Pringle. 2003. Macroinvertebrate biomonitoring in intermittent coastal plain streams impacted by animal agriculture. Journal of Environmental Quality 32:1036–1043.

De Haas, E. M., M. H. S. Kraak, A. A. Koelmans, and W. Admiraal. 2005. The impact of sediment reworking by opportunistic chironomids on specialised mayflies. Freshwater Biology 50:770–780.

Denver, J. M., S. W. Ator, L. M. Debrewer, M. J. Ferrari, J. R. Barbaro, T. C. Hancock, M. J. Brayton, and M. R. Nardi. 2004. Water quality in the Delmarva Peninsula; Delaware, Maryland, and Virginia, 1999-2001. U. S. Geological Survey Circular 1228, Reston, VA.

Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: The need for a flexible asymmetrical approach. Ecological Monographs 67:345.

Dukes, M. D., and R. O. Evans. 2006. Impact of agriculture on water quality in the North Carolina Middle Coastal Plain. Journal of Irrigation & Drainage Engineering 132:250–262.

Dunne, E. J., and K. R. Reddy. 2005. Phosphorus biogeochemistry of wetlands in agricultural watersheds. *in* E. J. Dunne, K. R. Reddy, and O. T. Carton, editors. Nutrient management in agricultural watersheds: A wetlands solution. Wageningen Academic Publishers, Wageningen.

Feldman, D. L., J. L. Farris, M. T. Moore, and C. M. Cooper. 2010. A characterization of benthic macroinvertebrate communities in agricultural drainage ditches of the northeast Arkansas Delta, USA. Pages 17–35 *in* M. T. Moore and R. Kröger, editors. Agricultural Drainage Ditches: Mitigation Wetlands for the 21st Century. Research Signpost, Kerala, India.

Feminella, J. W., and C. P. Hawkins. 1995. Interactions between stream herbivores and periphyton: A quantitative analysis of past experiments. Journal of the North American Benthological Society 14:465–509.

Flint, O. 1958. The larva and terrestrial pupa of *Ironoquia parvula* (Trichoptera, Limnephilidae). Journal of the New York Entomological Society 66:59–62.

Fouss, J. L., and M. Sullivan. 2009. Agricultural drainage management systems task force (ADMSTF). Pages 4068–4077 *in* S. Starrett, editor. Proceedings, World Environmental and Water Resources Congress, 17-21 May 2009 Kansas City, MO. ASCE, Reston, VA.

Fox, J., and S. Weisberg. 2011. An {R} companion to applied regression. Sage, Thousand Oaks, CA.

Freckman, D. W., T. H. Blackburn, L. Brussaard, P. Hutchings, M. A. Palmer, and P. V. R. Snelgrove. 1997. Linking biodiversity and ecosystem functioning of soils and sediments. Ambio 26:556–562.

Gallon, C., L. Hare, and A. Tessier. 2008. Surviving in anoxic surroundings: how burrowing aquatic insects create an oxic microhabitat. Journal of the North American Benthological Society 27:570–580.

Gerino, M., G. Stora, F. François- Carcaillet, F. Gilbert, J.-C. Poggiale, F. Mermillod-Blondin, G. Desrosiers, and P. Vervier. 2003. Macro-invertebrate functional groups in freshwater and marine sediments: a common mechanistic classification. Vie Millieu 53:221–231.

Greenberg, A. E., editor. 1995. Standard Methods for the Examination of Water and Wastewater. 19th edition. American Public Health Association, Washington, DC.

Grumbles, B. H. 1991. Wetlands, drainage ditches, and the Clean Water Act. Journal of Soil and Water Conservation 46:174–177.

Guzman, J. A., G. A. Fox, R. W. Malone, and R. S. Kanwar. 2009. *Escherichia coli* transport from surface-applied manure to subsurface drains through artificial biopores. Journal of Environmental Quality 38:2412–2421.

Hagy, J. D., W. R. Boynton, C. W. Keefe, and K. V. Wood. 2004. Hypoxia in Chesapeake Bay, 1950-2001: Long-term change in relation to nutrient loading and river flow. Estuaries 27:634–658.

Herzon, I., and J. Helenius. 2008. Agricultural drainage ditches, their biological importance and functioning. Biological Conservation 141:1171–1183.

Hill, C. R., and J. S. Robinson. 2012. Phosphorus flux from wetland ditch sediments. Science of the Total Environment 437:315–322.

Holford, I. C. R., and W. H. Patrick. 1979. Effects of reduction and pH changes on phosphate sorption and mobility in an acid soil. Soil Science Society of America Journal 43:292–297.

Holomuzki, J. R., J. W. Feminella, and M. E. Power. 2010. Biotic interactions in freshwater benthic habitats. Journal of the North American Benthological Society 29:220–244.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3–35.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346–363.

Huang, L., S. Du, L. Fan, X. Lin, H. Wang, and Y. Zhang. 2011. Microbial activity facilitates phosphorus adsorption to shallow lake sediment. Journal of Soils and Sediments 11:185–193.

Huett, D. O., S. G. Morris, G. Smith, and N. Hunt. 2005. Nitrogen and phosphorus removal from plant nursery runoff in vegetated and unvegetated subsurface flow wetlands. Water Research 39:3259–3272.

Hunting, E. R., M. H. Whatley, H. G. van der Geest, C. Mulder, M. H. S. Kraak, A. M. Breure, and W. Admiraal. 2012. Invertebrate footprints on detritus processing, bacterial community structure, and spatiotemporal redox profiles. Freshwater Science 31:724–732.

ICID. 2010. Important data of ICID member countries. International Commission of Irrigation and Drainage.

Jaynes, D. B., T. C. Kaspar, T. B. Moorman, and T. B. Parkin. 2008. In situ bioreactiors and deep drain-pipe installation to reduce nitrate losses in artificially drained fields. Journal of Environmental Quality 37:429–436.

Karr, J. R., and E. W. Chu. 1998. Restoring life in running waters: better biological monitoring. Island Press, Washington, D.C.

Keitzer, S. C., and R. R. Goforth. 2013. Salamander diversity alters stream macroinvertebrate community structure. Freshwater Biology 58:2114–2125.

Kenney, M. A., A. E. Sutton-Grier, R. F. Smith, and S. E. Gresens. 2009. Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. Terrestrial Arthropod Reviews 2:99–128.

Kerrn-Jespersen, J. P., and M. Henze. 1993. Biological phosphorus uptake under anoxic and aerobic conditions. Water Research 27:617–624.

Kleinman, P. J. A., A. L. Allen, B. A. Needelman, A. N. Sharpley, P. A. Vadas, L. S. Saporito, G. J. Folmar, and R. B. Bryant. 2007. Dynamics of phosphorus transfers

from heavily manured Coastal Plain soils to drainage ditches. Journal of Soil and Water Conservation 62:225–234.

Kovacic, D. A., M. B. David, L. E. Gentry, K. M. Starks, and R. A. Cooke. 2000. Effectiveness of constructed wetlands in reducing nitrogen and phosphorus export from agricultural tile drainage. Journal of Environmental Quality 29:1262–1274.

Krantzberg, G. 1985. The influence of bioturbation on physical, chemical and biological parameters in aquatic environments: A review. Environmental Pollution Series A, Ecological and Biological 39:99–122.

Kristensen, E. 1988. Benthic fauna and biogeochemical processes in marine sediments: microbial activities and fluxes. *in* T. H. Blackburn and J. Sørensen,, editors. Nitrogen cycling in coastal marine environments. John Wiley and Sons Inc., Chichester [West Sussex]; New York.

Kröger, R., E. Dunne, J. Novak, K. King, E. McLellan, D. Smith, J. Strock, K. Boomer, M. Tomer, and G. Noe. 2013. Downstream approaches to phosphorus management in agricultural landscapes: Regional applicability and use. Science of the Total Environment 442:263–274.

Kröger, R., M. M. Holland, M. T. Moore, and C. M. Cooper. 2008. Agricultural drainage ditches mitigate phosphorus loads as a function of hydrological variability. Journal of Environmental Quality 37:107–13.

Kuhn, M., S. Weston, J. Wing, J. Forester, and T. Thaler. 2013. contrast: A collection of contrast methods.

Langheinrich, U., S. Tischew, R. M. Gersberg, and V. Lüderitz. 2004. Ditches and canals in management of fens: opportunity or risk? A case study in the Drömling Natural Park, Germany. Wetlands Ecology and Management 12:429–445.

Lavorel, S., and E. Garnier. 2001. *Aardvarck* to *Zyzyxia*– functional groups across kingdoms. New Phytologist 149:360–363.

Leader, J. W., E. J. Dunne, and K. R. Reddy. 2008. Phosphorus sorbing materials: sorption dynamics and physicochemical characteristics. Journal of Environmental Quality 37:174–181.

Lecerf, A., and J. S. Richardson. 2010. Biodiversity-ecosystem function research: Insights gained from streams. River Research and Applications 26:45–54.

Leslie, A. W., R. F. Smith, D. E. Ruppert, K. Bejleri, J. M. Mcgrath, B. A. Needelman, and W. O. Lamp. 2012. Environmental factors structuring benthic macroinvertebrate communities of agricultural ditches in Maryland. Environmental Entomology 41:802–812.

Lewandowski, J., C. Laskov, and M. Hupfer. 2007. The relationship between *Chironomus plumosus* burrows and the spatial distribution of pore-water phosphate, iron and ammonium in lake sediments. Freshwater Biology 52:331–343.

Light, T. S. 1972. Standard solution for redox potential measurements. Analytical Chemistry 44:1038–1039.

Lohrer, A. M., S. F. Thrush, and M. M. Gibbs. 2004. Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431:1092–1095.

Lott, J. N. A., I. Ockenden, V. Raboy, and G. D. Batten. 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Science Research 10:11–33.

Lund, D. D. 2011. Assessment of the effects of conservation practices on cultivated cropland in the Chesapeake Bay Region. U.S. Dept. of Agriculture, Natural Resources Conservation Service, Washington, D.C.

Mackie, G. L. 2004. Applied Aquatic Ecosystem Concepts. 2nd edition. Kendall Hunt Publishing, Dubuque, Iowa.

Malmqvist, B. 2002. Aquatic invertebrates in riverine landscapes. Freshwater Biology 47:679–694.

Malmqvist, B., R. S. Wotton, and Y. Zhang. 2001. Suspension feeders transform massive amounts of seston in large northern rivers. Oikos 92:35–43.

Maynard, J. J., A. T. O'Geen, and R. A. Dahlgren. 2011. Sulfide induced mobilization of wetland phosphorus depends strongly on redox and iron geochemistry. Soil Science Society of America Journal 75:1986–1999.

McCall, P. L., and J. B. Fisher. 1980. Effects of tubificid oligochaetes on physical and chemical properties of Lake Eerie sediments. *in* R. O. Brinkhurst and D. G. Cook, editors. Aquatic oligochaete biology. Plenum Press, New York.

McDowell, D. M., and R. J. Naiman. 1986. Structure and function of a benthic invertebrate stream community as influenced by beaver (*Castor canadensis*). Oecologia 68:481–489.

Mermillod-Blondin, F., F. François-Carcaillet, and R. Rosenberg. 2005. Biodiversity of benthic invertebrates and organic matter processing in shallow marine sediments:

An experimental study. Journal of Experimental Marine Biology and Ecology 315:187–209.

Mermillod-Blondin, F., M. Gerino, V. Degrange, R. Lensi, J.-L. Chasse, M. Rard, and M. C. des Chatelliers. 2001. Testing the functional redundancy of *Limnodrilus* and *Tubifex* (Oligochaeta, tubificidae) in hyporheic sediments: An experimental study in microcosms. Canadian Journal of Fisheries & Aquatic Sciences 58:1747–1759.

Mermillod-Blondin, F., D. Lemoine, J.-C. Boisson, E. Malet, and B. Montuelle. 2008. Relative influences of submersed macrophytes and bioturbating fauna on biogeochemical processes and microbial activities in freshwater sediments. Freshwater Biology 53:1969–1982.

Mermillod-Blondin, F., and R. Rosenberg. 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquatic Sciences 68:434–442.

Merritt, R. W., K. W. Cummins, and M. B. Berg, editors. 2008. An Introduction to the Aquatic Insects of North America. 4th edition. Kendall/Hunt Pub. Co., Dubuque, Iowa.

Meysman, F. J. R., J. J. Middelburg, and C. H. R. Heip. 2006. Bioturbation: a fresh look at Darwin's last idea. Trends in Ecology & Evolution 21:688–695.

Michaud, E., G. Desrosiers, F. Mermillod-Blondin, B. Sundby, and G. Stora. 2006. The functional group approach to bioturbation: II. The effects of the *Macoma balthica* community on fluxes of nutrients and dissolved organic carbon across the sediment–water interface. Journal of Experimental Marine Biology and Ecology 337:178–189.

Millard, C. J., P. F. Kazyak, and A. P. Prochaska. 2001. Caroline County results of the 1994-1997 Maryland Biological Stream Survey: County assessments. Chesapeake Bay and Watershed Programs Monitoring and Non-Tidal Assessment - EA-01-31.

Miltner, R. J., and A. E. T. Rankin. 1998. Primary nutrients and the biotic integrity of rivers and streams. Freshwater Biology 40:145–158.

Molles, M. 2012. Ecology: Concepts and Applications. 6th edition. McGraw-Hill Science/Engineering/Math, New York.

Montserrat, F., C. Van Colen, S. Degraer, T. Ysebaert, and P. M. J. Herman. 2008. Benthic community-mediated sediment dynamics. Marine Ecology Progress Series 372:43–59.

Moore, A. A., and M. A. Palmer. 2005. Invertebrate biodiversity in agricultural and urban headwater streams: implications for conservation and management. Ecological Applications 15:1169–1177.

Moore, J. W. 2006. Animal ecosystem engineers in streams. BioScience 56:237–246.

Moore, M. T., and R. Kröger, editors. 2010. Agricultural Drainage Ditches: Mitigation Wetlands for the 21st Century. Research Signpost, Kerala, India.

Needelman, B. A., P. J. A. Kleinman, J. S. Strock, and A. L. Allen. 2007a. Improved management of agricultural drainage ditches for water quality protection: An overview. Journal of Soil and Water Conservation 62:171–177.

Needelman, B. A., D. E. Ruppert, and R. E. Vaughan. 2007b. The role of ditch soil formation and redox biogeochemistry in mitigating nutrient and pollutant losses from agriculture. Journal of Soil and Water Conservation 62:207–215.

Nguyen, L., and J. Sukias. 2002. Phosphorus fractions and retention in drainage ditch sediments receiving surface runoff and subsurface drainage from agricultural catchments in the North Island, New Zealand. Agriculture, Ecosystems & Environment 92:49–69.

Nogaro, G., F. Mermillod-Blondin, F. François- Carcaillet, J.-P. Gaudet, M. Lafont, and J. Gibert. 2006. Invertebrate bioturbation can reduce the clogging of sediment: an experimental study using infiltration sediment columns. Freshwater Biology 51:1458–1473.

Nogaro, G., F. Mermillod-Blondin, B. Montuelle, J.-C. Boisson, and J. Gibert. 2008. Chironomid larvae stimulate biogeochemical and microbial processes in a riverbed covered with fine sediment. Aquatic Sciences 70:156–168.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. vegan: Community ecology package.

Painter, D. 1999. Macroinvertebrate distributions and the conservation value of aquatic Coleoptera, Mollusca and Odonata in the ditches of traditionally managed and grazing fen at Wicken Fen, UK. Journal of Applied Ecology 36:33–48.

Palmer, M. A. 1997. Biodiversity and ecosystem processes in freshwater sediments. Ambio 26:571–577.

Palmer, M. A., and C. M. Febria. 2012. The heartbeat of ecosystems. Science 336:1393–1394.

Pavelis, G. A. 1987. Farm drainage in the United States: History, status, and prospects. United States Department of Agriculture: Economic Research Service, Washington, DC.

Penn, C. J., R. B. Bryant, P. J. A. Kleinman, and A. L. Allen. 2007. Removing dissolved phosphorus from drainage ditch water with phosphorus sorbing materials. Journal of Soil and Water Conservation 62:269–276.

Penn, C. J., J. M. McGrath, and R. B. Bryant. 2010. Ditch drainage management for water quality improvement: ditch drainage treatment structures. *in* M. T. Moore and R. Kröger, editors. Agricultural Drainage Ditches: Mitigation Wetlands for the 21st Century. Research Signpost, Kerala, India.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Marti, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86–90.

Phillips, S. W., editor. 2007. Synthesis of U.S. Geological Survey science for the Chesapeake Bay ecosystem and implications for environmental management. U. S. Geological Survey Circular 1316, 63p.

Pinder, L. C. V. 1986. Biology of freshwater chironomidae. Annual Review of Entomology 31:1–23.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2014. nlme: Linear and Nonlinear Mixed Effects Models.

Ponnamperuma, F. N. 1972. The Chemistry of Submerged Soils. Pages 29–96 *in* N.C. Brady, editor. Advances in Agronomy. Academic Press.

Powell, G. E., A. D. Ward, D. E. Mecklenburg, and A. D. Jayakaran. 2007. Two-stage channel systems: Part 1, a practical approach for sizing agricultural ditches. Journal of Soil and Water Conservation 62:277–286.

Rabenhorst, M. C. 2006. Synthetic iron oxides as an indicator of reduction in soils (IRIS). Soil Science Society of America Journal 70:1227–1236.

Rabenhorst, M. C. 2009. Making soil oxidation–reduction potential measurements using multimeters. Soil Science Society of America Journal 73:2198–2201.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Richardson, C. J. 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. Science 228:1424–1427.

Roberts, D. W. 2013. labdsv: Ordination and multivariate analysis for ecology.

Roskosch, A., N. Hette, M. Hupfer, and J. Lewandowski. 2012. Alteration of *Chironomus plumosus* ventilation activity and bioirrigation-mediated benthic fluxes by changes in temperature, oxygen concentration, and seasonal variations. Freshwater Science 31:269–281.

Russell, M. J., D. E. Weller, T. E. Jordan, K. J. Sigwart, and K. J. Sullivan. 2008. Net anthropogenic phosphorus inputs: Spatial and temporal variability in the Chesapeake Bay region. Biogeochemistry 88:285–304.

Sharpley, A. N., T. Krogstad, P. J. A. Kleinman, and B. Haggard. 2007. Managing natural processes in drainage ditches for nonpoint source phosphorus control. Journal of Soil and Water Conservation 62:197–206.

Shigaki, F., P. J. A. Kleinman, J. P. Schmidt, A. N. Sharpley, and A. L. Allen. 2008. Impact of dredging on phosphorus transport in agricultural drainage ditches of the Atlantic Coastal Plain. Journal of the American Water Resources Association 44:1500–1511.

Simon, T. N., and J. Travis. 2011. The contribution of man-made ditches to the regional stream biodiversity of the new river watershed in the Florida panhandle. Hydrobiologia 661:163–177.

Skaggs, B. W., M. A. Brev, and J. W. Gilliam. 1994. Hydrologic and water quality impacts of agricultural drainage. Critical Reviews in Environmental Science and Technology 24:1–32.

Smith, D. G. 2001. Pennak's Freshwater Invertebrates of the United States. Fourth edition. John Wiley and Sons Inc., New York, NY.

Smith, D. R., and E. A. Pappas. 2007. Effect of ditch dredging on the fate of nutrients in deep drainage ditches of the Midwestern United States. Journal of Soil and Water Conservation 62:252–261.

Ståhl-Delbanco, A., and L.-A. Hansson. 2002. Effects of bioturbation on recruitment of algal cells from the "seed bank" of lake sediments. Limnology and Oceanography 47:1836–1843.

Stephens, W. W., M. T. Moore, J. L. Farris, J. L. Bouldin, and C. M. Cooper. 2008. Considerations for assessments of wadable drainage systems in the agriculturally dominated deltas of Arkansas and Mississippi. Archives of Environmental Contamination and Toxicology 55:432–441.

Stockdale, A., W. Davison, and H. Zhang. 2009. Micro-scale biogeochemical heterogeneity in sediments: A review of available technology and observed evidence. Earth-Science Reviews 92:81–97.

Stone, M. L., M. R. Whiles, J. A. Webber, K. W. J. Williard, and J. D. Reeve. 2005. Macroinvertebrate communities in agriculturally impacted Southern Illinois streams: patterns with riparian vegetation, water quality, and in-stream habitat quality. Journal of Environmental Quality 34:907–917.

Stribling, J. B., B. K. Jessup, J. S. White, D. Boward, and M. Hurd. 1998. Development of a benthic index of biotic integrity for Maryland streams. Chesapeake Bay and Watershed Programs Monitoring and Non-Tidal Assessment - EA-98-3.

Strock, J. S., C. J. Dell, and J. P. Schmidt. 2007. Managing natural processes in drainage ditches for nonpoint source nitrogen control. Journal of Soil and Water Conservation 62:188–196.

Strock, J. S., P. J. A. Sleinman, K. W. King, and J. A. Delgado. 2010. Drainage water management for water quality protection. Journal of Soil and Water Conservation 65:131–1336.

Thomas, D. L., C. D. Perry, R. O. Evans, F. T. Izuno, K. C. Stone, and J. W. Gilliam. 1995. Agricultural drainage effects on water quality in southeastern U.S. Journal of Irrigation and Drainage Engineering 12:277–282.

Tilman, D. 1999. Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. Proceedings of the National Academy of Sciences 96:5995–6000.

Turner, A. M., and J. C. Trexler. 1997. Sampling aquatic invertebrates from marshes: evaluating the options. Journal of the North American Benthological Society 16:694–709.

United Nations. 2004. World Population to 2300. United Nations Department of Economic and Social Affairs, New York.

Usborne, E. L., R. Kröger, S. C. Pierce, J. Brandt, and D. Goetz. 2013. Preliminary evidence of sediment and phosphorus dynamics behind newly installed low-grade weirs in agricultural drainage ditches. Water, Air and Soil Pollution. 224:1–11.

US EPA. 2002a. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Environmental Protection Agency, Office of Water, Washington, D.C.

US EPA. 2002b. National Summary of Impaired Waters and TMDL Information. http://iaspub.epa.gov/waters10/attains_nation_cy.control?p_report_type=T#imp_water_by_state.

US EPA. 2009. National Water Quality Inventory Report to Congress. http://water.epa.gov/lawsregs/guidance/cwa/305b/.

U.S. Geological Survey. 1999. The quality of our nation's waters-nutrient and pesticides. U.S. Geological Survey Circular 1225:1–82.

Usio, N., and C. R. Townsend. 2004. Roles of crayfish: Consequences of predation and bioturbation for stream invertebrates. Ecology 85:807–822.

van de Bund, W. J., W. Goedkoop, and R. K. Johnson. 1994. Effects of deposit-feeder activity on bacterial production and abundance in profundal lake sediment. Journal of the North American Benthological Society 13:532–539.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Review of Ecology, Evolution, and Systematics 33:341–370.

Vasilas, L. M., G. W. Hurt, and C. Noble. 2010. Field indicators of hydric soils in the United States a guide for identifying and delineating hydric soils. U. S. Dept. of Agriculture, Natural Resources Conservation Service, Washington, D.C.

Vaughan, R. E., B. A. Needelman, P. J. A. Kleinman, and A. L. Allen. 2007. Spatial variation of soil phosphorus within a drainage ditch network. Journal of Environmental Quality 36:1096–104.

Vaughan, R. E., B. A. Needelman, P. J. A. Kleinman, and M. C. Rabenhorst. 2008. Morphology and characterization of ditch soils at an Atlantic Coastal Plain farm. Soil Science Society of America Journal 72:660–669.

Vaughn, C. C. 2010. Biodiversity losses and ecosystem function in freshwaters: Emerging conclusions and research directions. BioScience 60:25–35.

Venterink, H. O., T. E. Davidsson, K. Kiehl, and L. Leonardson. 2002. Impact of drying and re-wetting on N, P and K dynamics in a wetland soil. Plant and Soil 243:119–130.

Vepraskas, M. J., and S. P. Faulkner. 2001. Redox chemistry of hydric soils. Pages 85–105 *in* J. L. Richardson and M. J. Vepraskas, editors. Wetland Soils: Genesis, Hydrology, Landscapes, and Classificiation. CRC Press L.L.C.

Verdonschot, P. F. M. 1987. Aquatic oligochaetes in ditches. Hydrobiologia 155:283–292.

Verdonschot, P. F. M., and L. W. G. Higler. 1989. Macroinvertebrates in Dutch ditches: A typological characterization and the status of the Demmerik ditches. Hydrobiological Bulletin 23:135–142.

Verdonschot, R. C. M., H. E. Keizer-Vlek, and P. F. M. Verdonschot. 2011. Biodiversity value of agricultural drainage ditches: a comparative analysis of the aquatic invertebrate fauna of ditches and small lakes. Aquatic Conservation: Marine and Freshwater Ecosystems 21:715–727.

Wagner, R. 1991. The influence of the diel activity pattern of the larvae of *Sericostoma personatum* (Kirby & Spence) (Trichoptera) on organic matter distribution in stream-bed sediments — a laboratory study. Hydrobiologia 224:65–70.

Wallace, J. B., and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem functions. Annual Review of Entomology 41:115–139.

Wallace, J. B., J. R. Webster, and T. F. Cuffney. 1982. Stream detritus dynamics: regulation by invertebrate consumers. Oecologia 53:197–200.

Walshe, B. M. 1947. Feeding mechanisms of *Chironomus* larvae. Nature 160:474.

Webb, A. P., and B. D. Eyre. 2004. Effect of natural populations of burrowing thalassinidean shrimp on sediment irrigation, benthic metabolism, nutrient fluxes and denitrification. Marine Ecology Progress Series 268:205–220.

Wellborn, G. A., D. K. Skelly, and E. E. Werner. 1996. Mechanisms creating community structure across a freshwater habitat gradient. Annual Review of Ecology and Systematics 27:337–363.

Werner, I., D. A. Markiewicz, K. Goding, and K. Reece. 2010. Benthic macroinvertebrate communities in ephemeral agricultural drainage ditches of

California's Central Valley. Pages 1–15 *in* M. T. Moore and R. Kröger, editors. Agricultural Drainage Ditches: Mitigation Wetlands for the 21st Century. Research Signpost, Kerala, India.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer New York.

Williams, P., M. Whitfield, J. Biggs, S. Bray, G. Fox, P. Nicolet, and D. Sear. 2003. Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biological Conservation 115:329–341.

Withers, P. J. A., and H. P. Jarvie. 2008. Delivery and cycling of phosphorus in rivers: A review. Science of the Total Environment 400:1–3.

Woodward, G., G. Papantoniou, F. Edwards, and R. B. Lauridsen. 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. Oikos 117:683–692.

Yamamoto, Y. 2010. Contribution of bioturbation by the red swamp crayfish *Procambarus clarkii* to the recruitment of bloom-forming cyanobacteria from sediment. Journal of Limnology 69:102–111.

Young, E. O., and D. S. Ross. 2001. Phosphate release from seasonally flooded soils: a laboratory microcosm study. Journal of Environmental Quality 30:91–101.